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H. P. BOWDITCH, M.D., BOSTON
R. H. CHITTENDEN, PH.D., NEW HAVEN
W. H. HOWELL, M.D., BALTIMORE
FREDERIC S. LEE, PH.D., NEW YORK
JACQUES LOEB, M.D., CHICAGO
W. P. LOMBARD, M.D., ANN ARBOR
W. T. PORTER, M.D., BOSTON

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ON THE PHYSIOLOGY OF THE *PLANARIA MACULATA*, WITH ESPECIAL REFERENCE TO THE PHENOMENA OF REGENERATION.

By CHARLES RUSSELL BARDEEN

(Associate in Anatomy, the Johns Hopkins University).

[*From the Physiological Department of the Marine Biological Laboratory at Woods Holl, Mass.*]

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I. Introduction.

THE following article records the results of an attempt to determine certain internal conditions of regeneration in the *Planaria maculata*. I undertook the work at the suggestion of Professor Jacques Loeb, to whom I am also indebted for valuable criticism during the progress of the investigation. I wish here to express my appreciation of the privileges extended to me at the Marine Biological Laboratory by Dr. Loeb and Dr. C. O. Whitman, and of the courtesy accorded me at the U. S. Fish Commission by Dr. H. C. Bumpus.

An interesting historical review of experimental work on planarians is given by Harriet Randolph (1897). During the latter part of the eighteenth century and the first half of the nineteenth a considerable number of investigators experimented on planarians. Shaw (1791), Draparnauld (1801), Dalyell (1814), Johnson (1822), Dugès (1828), Faraday (1832), and Darwin (1844) were among them. These investigators discovered and pictured most of the remarkable phenomena of regeneration in the planarian that have been described by more recent researchers.

Renewed interest in the subject was aroused by the work of Van Duyne (1896).[1] Van Duyne, at the suggestion of Professor

[1] Van Duyne: Archiv für die gesammte Physiologie, 1896, lxiv, p. 569.

Loeb, took up the subject from a new point of view, that of heteromorphosis, a term applied by Dr. Loeb to those phenomena of regeneration in which an organ is replaced by one physiologically and morphologically unlike it. Van Duyne was able to cause the production of new heads and tails on various cut surfaces, the new heads being formed at times posterior to the situation of the remaining original head, but he failed to produce true axial heteromorphosis, the production of a head in the place of a tail.

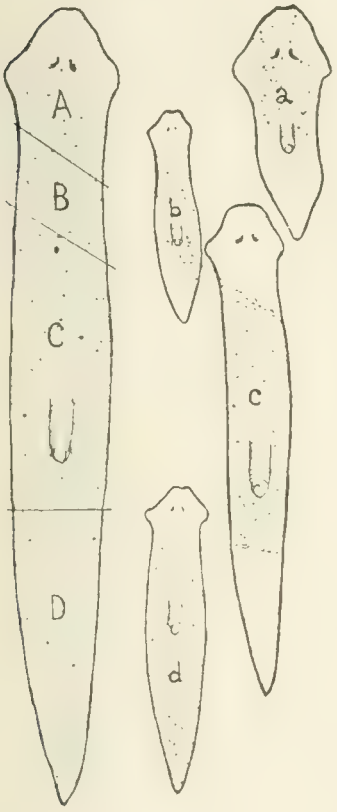


FIGURE 1. — Showing the regeneration of a new head from an anterior transverse surface, a new tail from a posterior transverse surface.

Since the appearance of Van Duyne's paper accounts of a number of researches on the subject have been published by Randolph (1897),[1] Morgan (1898, 1900),[2] and Lemon (1900).[3] Morgan in his last paper gives an instance of true axial heteromorphosis.

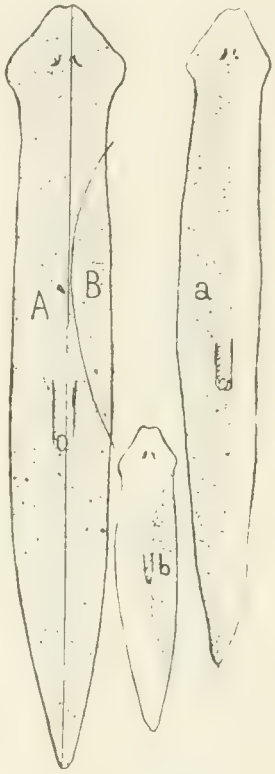


FIGURE 2. — Showing the regeneration of a new side, head, and tail from a lateral piece.

In brief, the various investigators have found that when the body of the worm is severed, an anterior transverse surface will readily regenerate a head (Fig. 1, B, b; C, c; D, d); a posterior transverse surface a tail[4] (Fig. 1, A, a; B, b; C, c); and a lateral piece a new head, side, and tail (Fig. 2, A, a; B, b). The

[1] RANDOLPH, HARRIET: Archiv für Entwicklungsmechanik der Organismen, 1897, v, p. 365.

[2] MORGAN, T. H.: Archiv für Entwicklungsmechanik der Organismen, 1898, vii, p. 365; 1900, x, p. 58.

[3] LEMON: Biological Bulletin, 1900, i, p. 193.

[4] The one known exception to this is that the head of an unknown species of Planaria was found by Morgan often to regenerate posteriorly a new head (*op. cit.*).

size of the worm thus regenerated varies in proportion to the size of the piece from which it is regenerated. The size of the smallest piece capable of regeneration has not yet been fully determined. Pieces cut from in front of the eyes will not regenerate (Morgan).

Instead of severing a piece from the body, the body may be split in an antero-posterior, a transverse, or an oblique direction. In these cases, if the wound is kept open, there is a tendency for the various parts to act as if the separation had been complete. In an antero-



FIGURE 3. — Effects of short longitudinal splitting in the median line.

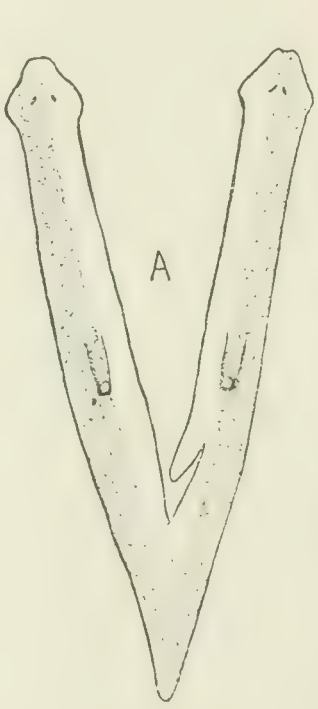


FIGURE 4.

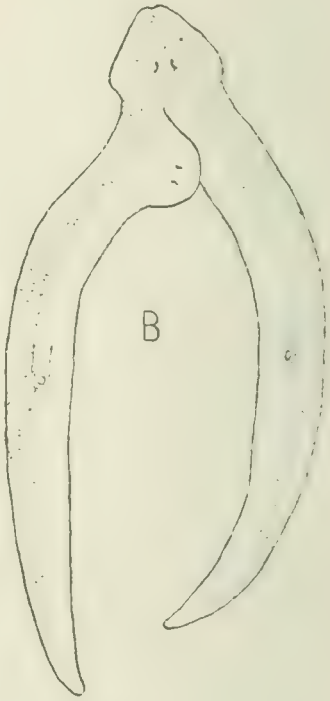


FIGURE 5.

Occasional effects of long longitudinal splits; in 4 an additional tail, in 5 an additional head is seen.

posterior splitting of the body each half head and side is transformed into a new whole head and "trunk," (Fig. 3, a); in a postero-anterior splitting each half tail becomes a new tail (Fig. 3, b). In the former case a new additional tail, in the latter a new additional head, may appear on the cut surface near the end of the split if the split be an extensive one, Figs. 4 and 5. If an oblique cut be made towards the median line from the side so as to isolate a strip of tissue, the "bud" (Lemon) thus produced may develop into a new head and trunk if directed anteriorly, and into a new tail if directed posteriorly (Fig. 6).

Loeb (1892)[1] has shown that in some animals gravity or contact or other external factors play a part in determining the nature of new organs produced. But he has also shown that in most animals external conditions have no power of specific determination. Van Duyne concludes his paper on heteromorphosis in Planaria by saying that the determination of the nature of the new-formed organs in the planarian must rest in internal, not in external, conditions.

Before proceeding to a description of the phenomena of regeneration experimentally produced in the fresh-water planarian, it seems best to give a brief account of the structure and bodily activities of the animal in so far as they can be followed under the microscope.

II. Anatomy.[2]

The *Planaria maculata* (Leidy, 1848), the most common of American fresh-water planarians, is found in great abundance in all the fresh-water ponds in the neighborhood of Woods Holl. It is found usually in groups of from three or four to fifteen or twenty on the under surface of loose stones lying in the shallow water near the edge of the pond.

External form. — An accurate description and a picture of its external form has recently been given by Woodworth (1897).[3] There is lacking a specific description of its internal structure, though Curtis (1900)[4] has given a valuable preliminary note on the structure and development of the reproductive organs.



Figure 6. — Development of lateral slips into head and tail pieces.

When resting the animal is usually considerably contracted in length, so that its outline is ovoid and often very irregular. When extended it becomes long, and, except for head and tail, of nearly equal width throughout its length, giving it the appearance of a bit of narrow ribbon. Extended for swimming the planarian in

[1] Loeb: Untersuchungen zur physiologischen Morphologie der Thiere. II. Organbildung und Wachsthum. Würzburg, 1892.

[2] The anatomy of several European fresh-water planarians has been described by Chichkoff: Archives de biologie, 1892, xii, p. 435; Jijima: Zeitschrift für wissenchaftliche Zoologie, 1844, xl, p. 359; and by others.

[3] Woodworth: Bulletin of the Museum of Comparative Zoölogy, Harvard College, 1897, xxxi, i.

[4] Curtis: The Johns Hopkins University Circulars, 1900, xix, p. 56.

this vicinity varies in length up to 20 mm., but seldom exceeds 10. When extended the width of the worm is approximately one-eighth of its length and the thickness one-fourth of its width.

The head is somewhat pointed and is bi-lobed. Where the head joins the trunk there are two small muscular protrusions, auricular appendages, which the worm has the power of moving about and which represent probably tactile organs. The pharynx lies midway between the anterior and posterior extremities of the worm.

Surface. — The mottled pigmentation of its dorsal surface is characteristic. The pigment lies mainly below the basement membrane, and is lacking over a region immediately antero-lateral to the eyes and over the base of the "auricular" appendages. The ventral surface is usually without much pigmentation.

The surface of the body is covered by columnar epithelium. This is ciliated over the ventral surface of the body and the external surface of the pharynx. The epithelium of the dorsal surface and of the edges of the body seems to be without cilia save along the lateral margins of the head and in a small area lateral to the eyes. In this last region the cilia are very long and their motion can be most conveniently followed.

Rhabdites, rod-shaped bodies, the secretion of special cells lying below the basement membrane of the surface epithelium, are closely packed in between the epithelial cells of the dorsal surface. Often they seem to lie within the cells also, but this has been disputed (Woodworth, 1891).[1] Owing mainly to the refraction and light dispersion caused by the rhabdites, the internal structures of the living worm may best be studied through the ventral surface, where the rhabdites are few in number and the pigmentation is less.[2]

Musculature. — The epithelium rests on a basement membrane. Below this lies the *muscular coat.* The latter completely encloses the animal. It is better developed on the ventral than on the dorsal surface. The two surfaces are connected by many dorso-ventral bands of musculature, which run both perpendicularly and obliquely

[1] Woodworth: Bulletin of the Museum of Comparative Zoology, Harvard College, 1891, xxi, 1.

[2] This is easily done by placing the worm in a drop of water on a cover-glass, and then inverting the cover-glass over a cell the margins of which do not touch the drop on the cover slip. If it is desired to flatten the worm the cover slip may be placed directly on a slide. The smaller the amount of water between cover-glass and slide the greater is the pressure on the worm.

and are inserted into or just below the basement membrane. Figure 7 illustrates longitudinal and cross sections through the musculature. The musculature constitutes not only an organ of contraction, but also, together with the basement membrane, the supporting framework of the animal. The main muscle coat, where well developed, is composed of the following layers from without inwards: (1) longitudinal, (2) transverse, (3) diagonal, (4) longitudinal.

The pharynx rests in a pocket, the lateral and posterior walls of which are composed mainly of dorso-ventral muscle bands. The ventral and dorsal walls of the pharyngeal pocket are thin, being composed mostly of the dermo-muscular coat which encloses the animal. The pharyngeal pocket is lined by a flattened non-ciliated epithelium.

At the anterior end of the pharyngeal pocket the pharynx is inserted into the body wall (Fig. 7). The union is effected mainly by

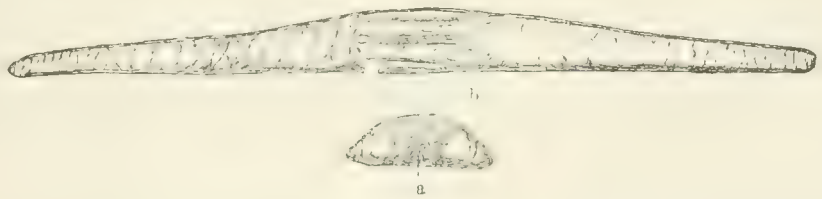


FIGURE 7. — Longitudinal and transverse sections through the middle line to show diagrammatically the ventral, dorsal, and pharyngeal musculature and the dorso-ventral bands. In the cross section a shows the plane of the longitudinal section. In the longitudinal section b shows the plane of the cross section.

an extension of the longitudinal musculature of the pharynx, which here radiates outwards. Additional strength is added by the dorso-ventral musculature.

The musculature of the pharynx consists essentially of two longitudinal tubes, an outer and an inner, together with a considerable amount of scattered muscular bundles, mainly longitudinal and radial, lying between. The outer muscular tube lies immediately below the basement membrane of the surface epithelium. It is composed of an external longitudinal and an inner circular layer of muscle fibres. The inner muscular tube surrounds the lumen of the œsophagus, and like the outer tube it is composed of longitudinal and circular layers. Many radial bands of muscle connect the two muscular tubes, and running between the two are longitudinal bundles of muscle. For the rest, the substance of the pharynx is made up of "parenchymal" and glandular cells.

The musculature of the body walls is continuous at the base of the pharynx with the outer tubular musculature of the pharynx and with the scattered longitudinal bundles.

The muscle fibres are not cross striated. Many of them are branched at the end. The nuclei of the muscle cells are not readily stained.

The muscular framework of the body gives form to the animal and support to the nervous, intestinal, reproductive and secretory apparatus, and to a loose "parenchyma." There is no true body cavity.

Nervous system. — The central nervous system lies immediately upon the ventral musculature. It consists of two nerve cords which run nearly the entire length of the animal. They are united by a dense commissural band at their anterior ends, and by numerous small commissural bands throughout their length (Fig. 8).

The nerve cords are composed of a network of bundles of non-medullated nerve fibres and of nerve cells placed singly or in groups

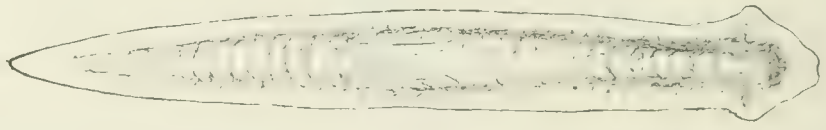


FIGURE 8. — Showing the distribution of the main nerve-fibre bundles. The cell bodies lie in the interstices and internodes. The eyes are placed relatively too far anterior.

either external to the bundles of fibres or surrounded by them. In the region of the head the bundles of fibres composing the nerve cords and the main commissural band are numerous and large, and they are surrounded by a very dense mass of cells. This part of the central nervous system has been dignified by being called a brain.

Some doubt exists as to whether the dense mass of cells is composed of nerve cells or of parenchyma cells (Woodworth, 1891).[1] In general it is very difficult in this animal to distinguish nerve cells from parenchyma cells, and in many instances it is impossible to tell them apart without the use of special nerve methods. Professor A. B. Morrill, who at present is working upon this subject, tells me that he finds this planarian unusually refractory to differential stains. The true nerve cells seem to be mainly bi-polar in form. Some are apparently multi-polar. It is probable that a certain number of the smaller spindle-shaped cells seen within and upon the bundles of

[1] WOODWORTH : *Loc. cit.*

nerve fibres are of a protective, possibly nutritive, nature, similar to the cells which incompletely ensheath the bundles of fibres in the sympathetic system of the vertebrates.

The nerve cords decrease in size from their anterior to their posterior extremities. They give rise to the peripheral nerves. In the region of the head these radiate out from the "brain" towards the margin of the head. A special nerve bundle (the optic nerve) may be traced to each eye, and a special bundle to the region which lies below the non-pigmented area at the base of the auricular appendages.

Passing posteriorly from the region of the head one finds numerous branches reaching towards the median line and laterally from each nerve cord. There seems to be no marked regularity in the intervals at which the peripheral nerves leave the main nerve cords.

At the base of the pharynx the nerve cords usually give off a good-sized nerve on each side, which enters the pharynx and runs towards its extremity. There is a well marked nerve ring about the orifice of the pharynx.

The peripheral nerves contain not only bundles of nerve fibres, but often nerve cells, and the sheath cells mentioned above.

From the peripheral nerves there probably arises an extensive peripheral nerve plexus for the supply of the musculature of the body and of the skin and possibly of some other organs. This plexus has not yet, however, been successfully demonstrated in the planarian. Details concerning the relation of the elements of the central nervous system are also needed (Lang, 1879).[1]

The eyes lie between the brain and the dorsal musculature. They are simple in structure. Each consists essentially of a cup composed of pigment cells and of cells of special sense. The bodies of the latter lie lateral to the pigment cup. Each cell sends a rod-like process into the pigment cup, and a nerve process into the anterior end of the nerve cord of the same side of the body. For details concerning the structure of the planarian eye the reader is referred to the excellent articles of Erick Jänichen (1896)[2] and Hesse (1897).[3]

Intestinal system. — The intestinal system is essentially that typical of the triclads. In the normal worm extended for easy swimming

[1] Lang: Mittheilungen aus dem zoologischen Station zu Neapel, 1879, i, p. 460.

[2] Jänichen, Erick: Zeitschrift für wissenschaftliche Zoologie, 1896, lxii, p. 259.

[3] Hesse: Zeitschrift für wissenschaftliche Zoologie, 1897, lxii, p. 527.

the pharynx lies midway between the anterior and the posterior extremities of the animal. From the pharynx the main branch of the intestine passes forwards in the median line to terminate in the region between the eyes (Fig. 9). Near the origin of this main axial trunk a large branch given off on each side passes laterally around the pharyngeal pocket, and then courses towards the tip of the tail. Before reaching the tip it often anastomoses with its fellow of the opposite side.

While there is a considerable degree of regularity about these main branches, the greatest irregularity prevails in the course and distribution of the secondary branches. These are given off both by the main axial gut and by the main branches to the tail. They may have an antero-lateral or a postero-lateral direction, though the former prevails in the region anterior to the base of the pharynx, the latter in the region posterior to the base of the pharynx. From the secondary branches tertiary branches are given off and frequently from



FIGURE 9. — Showing the branching and general relations of the intestines.

these still smaller branches. There is a considerable degree of anastomosis between these smaller peripheral branches. The result of this distribution of the intestinal branches is that every part of the body save the pharynx and the region of the head anterior to the eyes is brought into close proximity to the intestines. As in the case of the smaller branches of the arteries, veins, and nerves in the mammalian body, the distribution of the smaller intestinal branches here seems to be according to the needs of the part rather than to follow a strict inherited type. Thus when one branch for any reason fails to develop, neighboring branches grow out to supply the territory deprived of its more common source of supply.

The intestinal epithelium is composed in the main of large cylindrical cells very irregular in outline (Fig. 10). The cells may be spindle-shaped, narrow, and thread-like, or they may be broad and barrel-shaped, according to the amount of ingested food particles which they contain. The nuclei are arranged in rows. From time to time epithelial cells are cast into the lumen of the intestines. The active cells seem, however, all to be attached by a process to a base-

ment network composed of extensions from the bases of the epithelial cells. At the base of the epithelium spindle-shaped cells form a layer complete except for the prolongations of the larger cells above. These spindle cells differ slightly in form and staining power from the cells above and represent the younger cells from which the others are renewed.

The parenchymal canals in which the intestines lie have sharply defined walls composed of an interweaving network made up of the processes of the parenchymal cells. The epithelium is but loosely attached to the walls of the parenchymal canals. It is possible that fluids containing the digested food-stuffs circulate between the base of the epithelium and the lining of the parenchymal canals.

At the entrance of the intestines into the pharynx the tall columnar epithelium of the intestines passes over gradually into an epithelium of a lower type, in the lumen of the pharynx the cells become still smaller, and finally at a varying distance from the mouth of the pharynx they become ciliated. The great distensibility to which the pharynx is subject causes the epithelial surface to lie in folds when the pharynx is resting in the pharyngeal pocket.



FIGURE 10. — Showing the relation of the intestinal wall to the surrounding parenchyma.

Reproductive system. — The reproductive organs were wanting in most of the specimens with which I experimented. I shall therefore not attempt to describe them here. According to Curtis (1900) [1] the reproductive organs develop in the otherwise mature animals during the winter. In the spring these planarians reproduce sexually. As the summer comes on sexual reproduction gives way to reproduction by fission. During August and September it is practically impossible to find sexually mature animals in some of the ponds in the vicinity of Woods Holl, while in others sexually mature animals may be found throughout the season. For this information as well

[1] CURTIS: *Loc. cit.*

as for much else concerning the structure and habits of planarians in the vicinity of Woods Holl, I am indebted to Mr. Winterton C. Curtis. An excellent brief account of the anatomy and development of the reproductive organs of Planaria maculata will be found in his paper to which I have referred above.

Glands and parenchyma. — The space between the intestinal canals, the nervous system, the reproductive organs (when present), and the muscular framework of the animal is filled up by "parenchyma." In the parenchyma solitary gland-cells may be distinguished. These are of three distinct types. (1) Below the surface musculature, especially in the dorsal region, large cells may be seen, in the protoplasm of which rhabdites have been secreted. These cells are connected by a process with the surface of the body. (2) Throughout the parenchyma near the surface of the body but especially along the sides of the body and in the ventral region just behind the head, cells may be found the protoplasm of which is filled with granules staining a dark blue in hæmatoxylin. These are mucous cells. It is questioned whether the secretion of these cells reaches the surface of the body through processes of the cell connected with the body, or interstitially (Chichkoff, 1892).[1] (3) In the parenchyma of the œsophagus and in the vicinity of the main intestinal trunks large granular cells may be seen which stain intensely in Congo red. It is probable that these secrete a substance that enters the intestines.

In addition to the secretory cells just described it is probable that there exists an excretory system of the flame-cell, cell-chain type. Chichkoff pictures this for several species of planarians. I have not been able to see it in the somewhat opaque Planaria maculata.

Aside from the gland cells just described, which, properly speaking, perhaps do not belong to the parenchyma, the latter is made up of cells the nuclei of which stain intensely. The cell boundaries are not distinct, but for the most part the cells seem to belong to the branched connective tissue type.

III. Physiology.

I have given a more extended account of the anatomy of the planarian than I should were it not that the published accounts are scattered and the text-book accounts incomplete. Of the physiology of the planarian far less is known.

[1] Chichkoff: Archives de biologie, 1892, xii, p. 435.

It is convenient to separate the activities of an animal into two groups, those through which it is put into specific relation with its environment and those which subserve the internal functions of respiration, nutrition and growth. It is of course impossible to draw a sharp line between the two.

To the former group belong sensation, movement, and those nervous activities which correlate them. We shall take these up in the order mentioned.

Environmental activities. — *Sensation.* — The planarian is sensitive to light and to contact. Loeb (1894) [1] in an interesting paper has shown that the planarian reacts to light even when its head, including brain and eyes, has been removed. I have myself found that small pieces of the planarian, if capable of free movement, will show the same reaction. The planarian, Loeb showed, is not either positively or negatively heliotropic in the strict sense. Instead, it so reacts to light that when it comes to rest after moving about in a dish it stops at a place where the light is subdued, at the sides of a dish in front of a window for instance. In nature it rests preferably on the under surface of a projecting piece of stone. Further than this nothing is known of its "visual" power. The animal seems to move about more by night than by day.

The susceptibility to light is apt to become lost if the worms are kept in captivity. It is interesting to note in this connection that Chichkoff has found that the pigment of many planarians is much altered when these planarians are exposed to direct sunlight. It tends to disappear.

To touch, the planarian is very sensitive, apparently over the whole surface of its body. Not only does the planarian react so as to withdraw from an unpleasant foreign body, such for instance as a pin-point, but it also shows a certain form of stereotropism. If placed on the bottom of a glass dish containing water it will swim or crawl about until it reaches the side of the dish. It will then crawl up the side of the dish and come to rest in a vertical position. If, however, a piece of glass be placed over the dish so as to touch the surface of the water, a large number of the worms will follow up the side of the dish until they come to the piece of glass, and thence out upon its under surface, coming to rest in a horizontal position with the back towards the ground. Some planarians which I placed in

[1] Loeb: Archiv für die gesammte Physiologie, 1894, lvi, p. 247.

a vial of boiled water in order to test the effects of a lack of oxygen upon them, crawled toward the cork if the cork end was above, and toward the bottom of the vial if it was placed upside down. Although there was presumably more air near the cork than elsewhere in the vials, the worms all remained at the glass end of a vial left upside down until they died of asphyxiation.

If several planarians are placed in a small amount of water at the bottom of a bottle and the bottle is turned bottom up they will crawl down the sides of the flask back into the water. One might here suspect a specific attraction of the water on the planarian. But if the planarian is placed in a drop of water on a glass slide and the slide is held vertically the planarian will crawl downwards in the direction taken by the drop of water. It is apparent that the mechanical stress of the drop of water is such as to cause the planarian to crawl downwards. Occasionally both in the inverted bottle and on the glass slide the planarian seeks to proceed in a direction opposite to or different from that given the drop of water by gravity. But such counter-movement is seldom long maintained.

The cilia are supposed to play a part in the sense of touch.

I have been unable to satisfy myself that the worm is sensitive to anything but light and contact. It might naturally be supposed that taste should be also included. Yet I have repeatedly found that worms which have been kept in pure rain water for a week or two, and were thus in a hungry condition, would remain unmoved by the presence close by their side of a piece of fresh snail, a food much prized by them. If one of these worms was then removed to a slide and placed on its back in a drop of water too small completely to immerse it, it would as a rule soon protrude its pharynx, in search possibly of water. On the pharynx thus extended it was easy to place a bit of snail. This then would be quickly swallowed and as much more as could be given it, until the worm was swollen nearly to bursting with food. The pharynx will refuse to transfer into the intestines pieces of carmine or other hard particles, and so far as I am able to judge from incomplete experiments, peristaltic swallowing movements are set up in it only by soft pulpy objects, like the body of a snail.

The region just posterior to the auricular appendages has a rich nerve supply. Whether it is possessed of any organs of special sense is not known.

Movements. — By movement an animal may either be placed in

a different attitude towards the environment in which it remains, or it may be transferred by locomotion to another environment. We may first consider the locomotion of the planarian.

This takes place in one of two ways, either by means of contact with a solid body, or without such contact. In the latter case the simple action of the cilia on the ventral surface of the body seems at times sufficient to propel the planarian. The body is at such times extended ribbon-like, with the ventral surface apparently slightly protruded. It is rare, however, that one cannot detect in the little animals thus moving gracefully through the water a slight waving movement of the sides of the body near the head that aids in propulsion. The action of the cilia can be most easily studied in the moving animal at the margin of the head antero-lateral to the eyes. When the animal is moving in its usual direction, forwards, the direction of the sweep of the cilia is caudal. When the head comes to rest the ciliary action becomes slower; occasionally it may even cease for a short time. When the movement of the head is in a direction towards the posterior end of the animal, as when, for instance, the head is suddenly drawn back from some object, the movement of the cilia is reversed. This reversed action may possibly be set up by the mechanical friction of the water.

The slight waving movements noted at the margins of the body during the gentle gliding of the animal through the water, become much more marked when the animal moves more swiftly. In the most violent swimming movements contraction waves of considerable size may pass along the body from head to tail. This movement may sometimes be seen especially well after splitting the tail of a worm. I have never seen a worm swim backwards, though frequently I have seen them crawl short distances backwards.

While the swimming of the planarian takes place by means of cilia supplemented by muscular activity, the progress of the worm in contact with a solid surface seems to be mainly, if not wholly, by means of muscular activity. In this progression the ventral surface of the worm may be held concave, thus bringing the margins of the body which are free from cilia into contact with the surface. By means of the secretions of the mucous glands the lateral edges take a firm hold of the surface on which they rest. The hold is next relaxed in the region of the head and this is extended forwards. The wave of extension gradually passes caudalwards, the surface contact being meanwhile released. By the time the worm is fully

extended the head has taken a new hold, not only by the edges, but also by the ventral surface. When the tail is relaxed the posterior part of the worm is brought forward by a sudden contraction of the whole worm. When excited to rapid movement the back is sometimes arched during the movement of contraction, after the manner of a leech. Backward movements of a nature the reverse of the normal forward movements are occasionally made by a worm.

In addition to these movements of locomotion the worm may twist its body in any direction, readily right itself if placed on its back, withdraw its head well within the neck region, protrude its auricular appendages, extend its œsophagus and stretch it in any direction, and perform numerous other movements. I have been able to distinguish no distinct correlation between the movements of the œsophagus and those of the worm as a whole. The œsophagus may be protruded not only while the worm is contracted, but also when it is in an expanded condition.

Central nervous system. — Through the central nervous system sensory stimuli give rise to movements, and the movements are correlated. In considering the anatomy of the central nervous system we have seen that it is comparatively simple in structure. It is so arranged that at any given level it may govern the activities of all parts posterior to that level. Thus in the normal worm a slight prick at the side of the body may give rise merely to a local reaction, and a retraction of the body at that point. If, however, the prick be repeated or made more severe, the whole worm may be set into movement and the local reaction be quite lost in the general movement. In this reaction of the whole worm movement nearly always begins at the head. In the swimming animal a wave of contraction may pass from head to tail. If crawling, the head first stretches forth, and then the rest of the body is extended in turn. On the other hand, longitudinal contraction seems usually to be sudden, and to affect the whole body nearly at the same time. If the body is cut in two at any level, the anterior end of each section acts the part of a general centre of co-ordination for the whole piece. As Loeb [1] has shown, the brain has no observable specific function in the planarian. If the nerve cords be severed by a cut through the ventral surface that does not extend completely through the animal, sensory impulses are not transmitted from the part posterior to the cut to the brain. On the contrary, stimulation of the posterior

[1] Loeb: *Loc. cit.*, 1894, p. 247.

piece causes a contraction and expansion in this piece only. However, waves of muscular movement may pass over the cut area from the anterior to the posterior piece. If the animal as a whole moves rapidly and then the anterior piece either stops or moves more slowly, the rapid movements in the posterior piece usually continue for a short time. If the nerves are cut posterior to the pharynx the muscular waves of the anterior piece are not transmitted to the posterior piece for some hours after the operation. Normal activity in the nervous system is restored if the severed cords are left in contact from 24 to 48 hours.

Internal activities. — *Deglutition, food dispersion, and defecation.* — Experimentation with the external activities of the animal is comparatively easy. Experimental study of the internal activities is much more difficult. In planarians without a developed reproductive system the grosser internal activities centre chiefly about the branched intestinal system. Food is passed into the main gut by peristaltic waves of the pharynx. By contraction of the body wall the food is thence passed into the caudal divisions of the gut and into all its branches. The swallowing of food and its distribution in the intestines may readily be followed by feeding the animal in the manner mentioned above (p. 14) and then placing it under the microscope. A good method is to place the animal in a dish of water on a cover glass and invert this over a dry cell. Small animals with slight pigmentation answer the purpose best. It is remarkable with what rapidity the food is evenly distributed in the intestines by means of the various movements of the body wall of the worm. Just what assistance is lent by the internal musculature of the worm, the dorso-ventral bands, I have been unable to follow. The food is, however, kept in an irregular kind of circulation, rendered possible by the numerous anastomoses of the more peripheral branches of the intestine.

I have made no experiments which have enabled me to determine whether digestion is carried on in part in the lumen of the intestine, but it seems probable that it is. In the main, however, the digestion is intracellular, and the intestinal cells after feeding may be seen filled with bits of food and with various refractive particles. Because of these strongly refractive particles it is possible to trace the outlines of the intestines in the living animals.

After the intestinal epithelial cells have become filled with food particles the food debris remaining in the lumen of the intestines is

cast forth. This process of defecation takes place by means of a series of sudden contractions of the whole animal while the lumen of the pharynx is held open. After this process has taken place the intestines are very sharply outlined by the food particles which give a marked color to the intestinal epithelium.

Digestion takes place rather slowly. By the end of a week after feeding, however, marked changes have taken place. The food has become converted into globular refractive particles which distend the intestinal epithelium much more than did the original particles of food. Some of these refractive bodies stain dark brown in an iodine solution, others are turned black by osmic acid, and some seem to be affected by neither reagent. The effect of the distention thus produced in the epithelial cells is to cause many of them to desquamate. The lumen of the intestines becomes filled with swollen cells resembling fat cells and the intestines are thereby so much distended that it becomes difficult to follow their outline. This intestinal matter seems partially to be absorbed into the tissues, partially to be evacuated from the animal.

While it is easy to trace the food into the cells of the intestine, it is exceedingly difficult to make out the processes whereby the food stuffs are transferred to the tissues and internal respiration is carried on. Within the parenchyma many spaces are seen in the meshwork, which doubtless serve for the flow of lymph through the tissues, a flow aided by the contractions of the body. The general distribution of the digestive apparatus likewise reduces the necessity for a complicated vascular system for the distribution of food stuffs. It is also possible that the space between the intestinal epithelium and the inner lining of the enteric canals of the parenchyma serves to convey fluids from one part of the body to another. The lumen of the intestine therefore serves as the region for the distribution of undigested food stuffs and of aerated water, the perienteric space may serve for the distribution of the digested food stuffs, and the intercellular spaces of the parenchyma for that of the tissue bathing lymph.

The intestinal epithelium serves not only to digest food, but also as a storehouse for digested food.

Respiration. — Respiration must be carried on in part through the fluids which enter the intestine, in part from the surface of the body where it is helped by the movement of the cilia.

I have been able to discover no rhythmic activity either in the pharynx or in the intestines. The facts, however, that the muscular

movements of the animal tend to occur in series of waves from the head to the tail, and that longitudinal extension from the tail is a slow movement, longitudinal contraction towards the head a rapid one, lead us to assume that rather definite currents of diffusion may be thus set up.

Whether during rest there is a diffusion of fluids for respiration and nutrition I have been unable to decide. I have seen occasional non-rhythmic movements of the pharynx in animals apparently otherwise perfectly at rest.

Excretion is carried in part through the intestines by the act of defecation. In part it is doubtless carried on by an excretory system opening on the surface.

IV. Physiology of Regeneration.

During the summer months, in many localities at least, the Planaria maculata multiplies almost exclusively by fission. In the great majority of instances, this fission takes place transversely across the body in the region just posterior to the pharyngeal pocket. Of the processes giving rise to this fission I have made no special study. The regeneration of normal worms from each of the two pieces resulting from this fission, however, I have studied, and have found it to correspond closely to the regeneration which occurs when the body is artificially divided in this region. Since, therefore, the phenomena of regeneration after the division of the body in this region are continually taking place under normal conditions during the summer months, it may be of interest to begin with a description of them. We shall afterwards follow the phenomena of regeneration occurring in pieces separated wholly or partially from other regions of the body.

A. Regeneration after Dividing the Body posterior to the Pharynx.

Regeneration in the tail piece after transverse section. — The phenomena of this process may be grouped as follows:

1. Partial closure of the wound through the action of the musculature.

2. Protection at the cut surface first by a transformation of all cells directly exposed to the water into mucoid cells and later by epithelium which extends outwards from the margins of the wound.

3. Accumulation of cells of embryonic type near the cut surface.

From the cell mass thus produced the musculature and parenchyma of the new head are differentiated.

4. Anastomosis or fusion of the anterior ends of the two main posterior intestinal branches so as to produce an axial gut, and the development of new branches from this.

5. Transformation of the tissue posterior to this gut into a new pharynx.

6. Growth forwards into the region of the new head of branches arising from the two lateral nerve cords, and the production of a new brain and eyes.

7. Further differentiation into a worm of normal proportions.

These phenomena are illustrated in Figs. 11, 12 and 13, and will now be taken up in detail.

1. If a cross section is made through the body of the planarian in a region posterior to the pharyngeal pocket (Fig. 11, A) the tail piece thus isolated makes at once a number of violent convulsive movements. In the main these are movements of longitudinal expansion and contraction, often of unequal force on the ventral or dorsal surface, so as to cause a bowing of the piece. After these more violent movements the tail piece usually crawls and swims about for a short time, and then comes into a state of rest on the bottom of the dish.

Meanwhile the circular and oblique musculature at the margin of the cut surface contract violently so as to bring about the condition shown in Fig. 11, B. The dorso-ventro-musculature near the cut edge also contracts; the cut surface is thus reduced to the smallest possible dimensions, while the tension under which the exposed tissues are placed serves as a mechanical hindrance to the entrance of fluids from without. The contractions of the animal have meanwhile forced into the semicircular space formed by the infolding of the edges of the wound a certain amount of parenchyma. From each of the cut enteric canals a short bit of intestine is likewise extruded.

2. Some of the cellular mass thus extruded into the semicircular space becomes separated from the animal and floats away. The greater part of it, however, remains in place. The cells immediately exposed to the water, both the parenchymal cells and the intestinal cells, undergo a mucoid degeneration, well seen in hæmatoxylin preparations, and this serves to protect the tissues until the wound is covered by epithelium.

From the margin of the intact epithelium a thin epithelial sheet

soon spreads over the exposed cell mass. As the cells wander out from the epithelial margin they assume a flattened instead of a columnar form, and thus cover the surface the more quickly. After the surface is covered by the flattened epithelium, cell multiplication is rapid until lateral pressure causes the cells once more to assume the columnar form. Soon hereafter they become ciliated.

When the regeneration of the head has considerably advanced, rhabdites are formed in rhabdite gland cells below the surface and then are forced into the surface epithelium, where they lie between and within the epithelial cells. This is especially marked on the dorsal surface.

3. Meanwhile there has been very rapid multiplication among the parenchyma cells. As this continues the infolded edges of the old tissue are gradually forced apart again (Fig. 11, B, C, D) and the cell mass extends forwards gradually, and assumes more and more the characteristics of a head. For a considerable time the animal retains the power of partially withdrawing the head and closing in the edges of the old tissue, so that one is often surprised at the development of the head when the animal is aroused from a state of contraction and proceeds to move about.

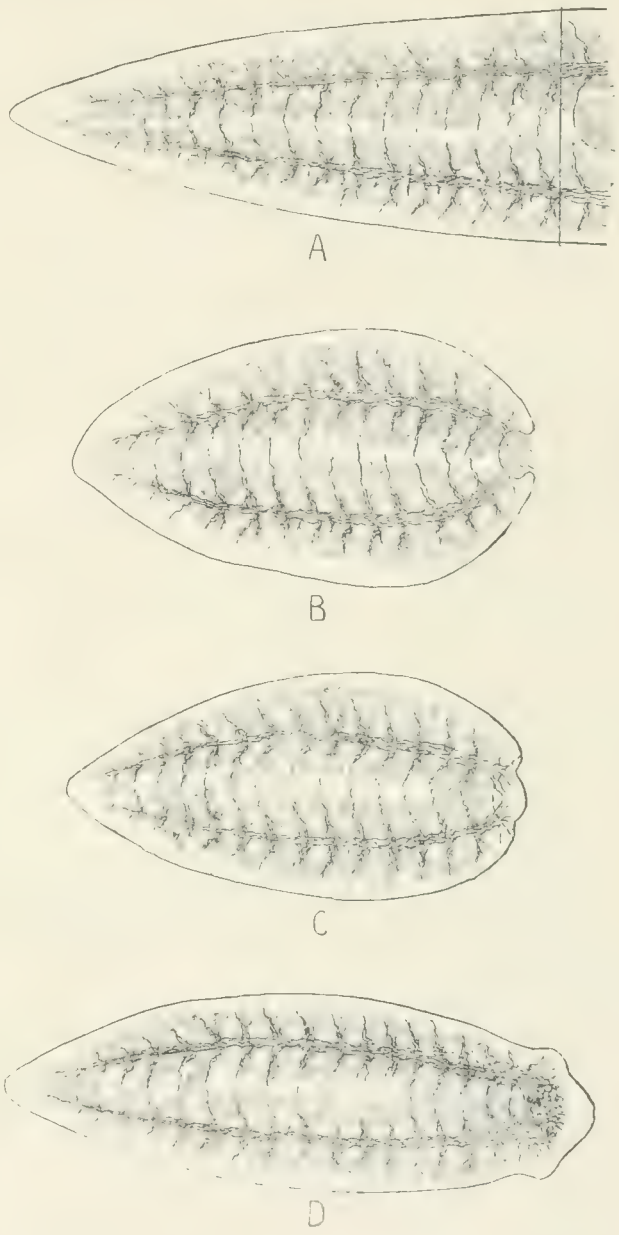


FIGURE 11. — Showing the main changes that take place in the tail region of the body after its removal. A. Before removal. B. Immediately after removal. C. About 3 days after removal. D. 10 days after removal.

I have not been able to follow accurately the differentiation of cells forming the dense mass which at first is formed in front of the cut surface into parenchyma cells and muscle cells.

4. Of vital importance in the determination of the arrangement of the elements going to form the head is the relation of the new cell mass to the intestines. By the force of the muscular contractions at the anterior end of the animal and by the extrusion of parenchyma cells the anterior extremities of the two main posterior intestinal branches are brought into close proximity. Within the first forty-eight hours fusion takes place so as to produce anteriorly in the piece a single axial gut (Fig. 11, C). Thus is produced an intestinal distribution similar to that formed in the adult worm, except that the axial gut is disproportionately short. The exact details in this process of intestinal fusion are difficult to follow, owing to the irregularity of form of the intestinal cells. That fusion of intestinal branches normally occurs is indicated by the frequent anastomosis seen between the intestinal branches of the normal worm. If a cut be made completely through a worm in a dorso-ventral direction so as to sever the intestines, and the cut surfaces be allowed to heal together again, the intestines will be found completely united within a few hours. It may be that the severance of the two posterior intestinal branches puts them into a condition which serves to promote anastomosis.

The cells of the newly formed intestinal branches contain bright refractive particles and but little pigment. It is therefore easy to distinguish them from the original intestinal branches, provided the worm has had food not too long before the operation. If worms are fed soon before operation so that the intestinal cells are filled with food stuffs, intestinal regeneration is very much retarded, owing probably to an interference with cell reproduction caused by the presence of contained masses of food stuffs. The pharynx in worms fed just before cutting develops in about seven days. In worms fed not too recently it develops usually within five days.

On the other hand if one operates on worms recently fed the digestive processes continue much as in normal worms. By the end of a week after feeding the intestines have become much distended and the intestinal contour is hard to follow. For this reason the clearest pictures are often obtained by operating on worms that have been long without food.

The regenerating worm makes movements of various sorts. The

effect of the contraction of the musculature posterior to the region of the axial gut is to exert a pressure on the two posterior branches of the intestine, and to drive any contents there contained into the axial gut (Fig. 12). On the other hand, contraction exerted on the parenchyma about the axial gut tends to drive the intestinal contents back to the posterior branches (Fig. 12). When contraction is simultaneous in both parts of the tail piece the intestinal contents are pressed with considerable force against the tissue lying posterior to the axial gut and between the lateral branches (Fig. 12). The movements tending to bring this about are similar to the movements causing defecation in the normal worm. They are not uncommon in the isolated tail piece, and may readily be followed if the tail be cut from a worm recently fed.

The first effects of this pressure are seen in a gradual prolongation backwards of the axial gut, so that this comes to assume more normal proportions in relation to the posterior intestinal branches.



FIGURE 12.—To show the direction taken by the intestinal contents when the body forcibly contracts.

New intestinal branches arise from the anterior extremity of the axial gut and extend into the region of the new head. In the outgrowth of a new intestinal branch one sees at first a bud-like bunch of cells extending outwards from the intestinal wall. This cell bunch soon assumes a cylindrical form, acquires a lumen, and takes on the morphological peculiarities characteristic of the planarian intestines.

The new head and the new pharynx of the worm are formed with relation to the axial gut. The head is formed symmetrically about the anterior extremity of the axial gut, the pharynx is formed at its posterior extremity. We shall now consider the latter process.

5. As the axial gut becomes prolonged posteriorly marked alterations may be noted in the tissue into which it is extending. These alterations become apparent about two days after the tail piece has been isolated. One first notices a number of cells undergoing retrograde metamorphosis. The protoplasm of these cells first becomes granular and then of a uniform consistency staining deeply in stains like Congo red. The nuclei break up. These alterations are seen both in muscle cells and in parenchyma cells. Soon a rapid cell

multiplication takes place in the latter, and in place of cells with extensive protoplasmic processes, we find small ovoid cells of an embryonic type densely packed together. The tissue posterior to the axial gut now appears quite dense. (Fig. 13, A).

The axial gut extends further posteriorly ending in an axial process beyond the place of anastomosis of the two posterior intestinal branches (Fig. 11, C). About this extension of the axial gut the embryonic tissue becomes especially dense (Fig. 13, B), and a space appears surrounding this denser tissue on all sides except that towards the axial gut. This space represents the pharyngeal pocket, and at first apparently has no communication with the surface of the body. The cell mass about the extremity of the axial gut represents the beginning of the pharynx. This rapidly extends its growth posteriorly, and the small round cells composing it become differentiated into the tissues characteristic of the pharynx. Fig. 13, C, represents the pharynx at the time of the formation of its lumen; Fig. 13, D, represents the pharynx protruding slightly through the opening into the pharyngeal pocket. This opening is formed by invagination of the external surface about the fourth to fifth day after the isolation of the tail piece.

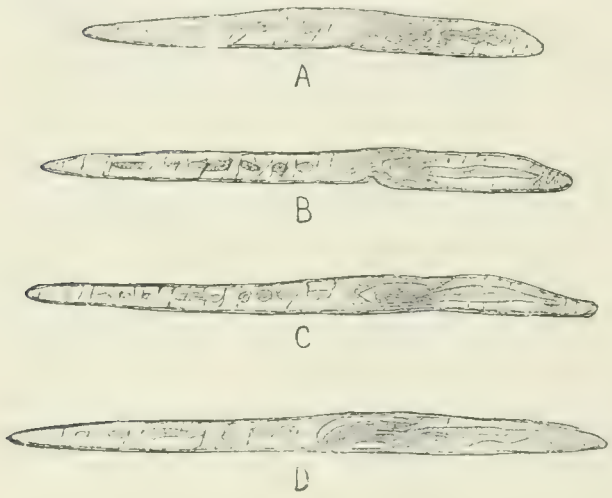


FIGURE 13. — Longitudinal sections through the axis of the regenerating tail piece, showing four stages in the formation of the pharynx in a tail piece. A. Formation of embryonic tissue posterior to the axial gut (about two days after isolation of the piece). B. Differentiation of embryonic tissue about the axial gut, and formation of pharyngeal pocket. C. Appearance of lumen in pharynx. D. Breaking through of pharyngeal pocket (about 5 days after isolation of the piece).

6. Meanwhile the nervous system has been growing rapidly and soon after the appearance of the pharynx the eye spots become well marked. Under normal conditions the pharynx appears several hours before the appearance of the eye spots.

By the contraction of the musculature of the anterior extremity of the piece the nerve cords have been brought nearer to one another. From each of them processes grow forth into the new

tissue. The largest of these grow towards the median line anterior to the median gut, and then from each side unite to form the main commissure of the new "brain" (Fig. 11, D).

The general stages in the production of a new brain have been described by Flexner (1898).[1] In the main I have been able to confirm the conditions found by Flexner, though I do not wholly agree with him in his deductions.

The first thing one notices about the cut end of the nerve cord is a granular degeneration of nerve fibres and of some cells. This is soon succeeded by a growth of bundles of nerve fibres into the newly formed parenchyma. Between these bundles of fibres many nerve cells can be seen. Mitosis is met with very rarely (Flexner was unable to find it) in these cells. I think, however, technique and not lack of cell division is responsible for this. Fibre bundles thus direct the way into the new tissue, while the new nerve cells wander along these to form ganglionic centres, whence new bundles of fibres are again sent forth. In its essentials, therefore, the growth of the nervous system here corresponds closely with the growth of the sympathetic system in the vertebrates.[2] Whether the new nerve cells arise from well developed neurons or from an embryonic form of nervous tissue existing in the developed nervous system of the planarian, I cannot say.

The regeneration of the eye has been carefully studied by Erick Jänichen (1896).[3] So far as my studies have extended they fully confirm Jänichen's work. I am inclined to think, however, that the "retinal" cells of the eye wander forward from the central nervous system, and are not developed from parenchymal cells.

7. By the fourth or fifth day after the tail piece has been isolated we have a worm essentially complete. The head is somewhat small, the eyes are not well developed, though the pigment of the pigment cup makes them visible, and the pharynx is not completely differentiated and is situated rather too far anteriorly. The worm, however, rapidly assumes normal proportions. The new worm is slightly narrower than was the parent worm in the region through which the cut passed. The pharynx comes to occupy a region midway between the two extremities, owing in part to extension backwards of the axial gut, in part to a growth forwards of the

[1] Flexner: Journal of morphology, 1898, xiv, p. 337.

[2] His: Archiv für Anatomie und Physiologie, 1897, p. 137.

[3] Jänichen: *Loc. cit.*

head. In from two to three weeks the new worm may be of perfectly normal proportions.

The newly regenerated areas remain long but slightly pigmented.

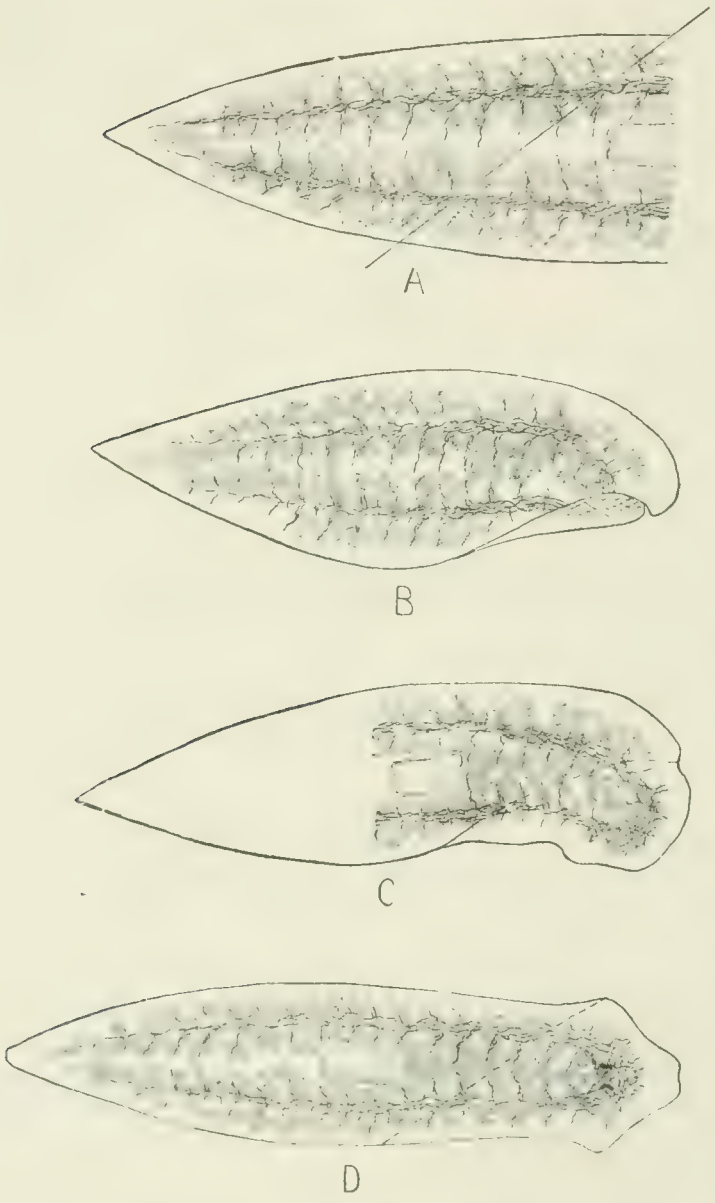


FIGURE 14. — To illustrate the growth of a new head and pharynx from a tail piece after oblique section. A. Place of section. B. 24 hours after isolation. C. 4 to 5 days after isolation. D. About three weeks after isolation.

The processes of regeneration just described are essentially the same whether the tail piece is severed from the body very near the pharynx or near the posterior extremity of the worm. Even very short tail pieces will regenerate, but the process is then somewhat slower.

Regeneration in the tail piece after oblique section. — When the tail is severed from the body by an oblique section the processes of regeneration are essentially the same as after transverse section, but there is some difference in detail (Fig. 14).

1. The cut surface is much greater than after transverse section, and can therefore not be as closely contracted by the action of the body musculature. On the long side of the tail piece the edge curls in towards the cut surface, and on the short side the edge bends in, showing that the cut surface has been reduced to the smallest possible dimensions by muscular activity (Fig. 15, B).

2. The anterior end of the main posterior intestinal trunk on the

longer side of the piece becomes the axial gut (Fig. 15, B). The shorter posterior intestinal trunk becomes united to this by anastomosis (Fig. 15, B). Just posterior to the region where this anastomosis occurs a new pharynx becomes developed in a manner similar to that described in tail pieces isolated by transverse section.

3. Embryonic tissue is produced throughout the length of the cut surface, but in greatest abundance about the tip of the axial gut, where it becomes differentiated into a new head. The longitudinal axis of the head at first forms an obtuse angle with the long axis of the body (Fig. 15, C), but this is gradually overcome by a greater production of tissue on the short side of the cut piece. Finally the longitudinal axis of the head is brought into line with the long axis of the body. As the new tissue is formed from which the head and side of the body are differentiated, the axial gut sends branches into it. Unequal growth continues until there is approximately the same amount of tissue on the one side of the axial gut as on the other, and until the tip of the head is approximately as far from the pharyngeal pocket as the tip of the tail. After this the animal grows symmetrically.

4. The nervous system develops from the old nervous system in a manner similar to that described for tail pieces isolated by a transverse cut.

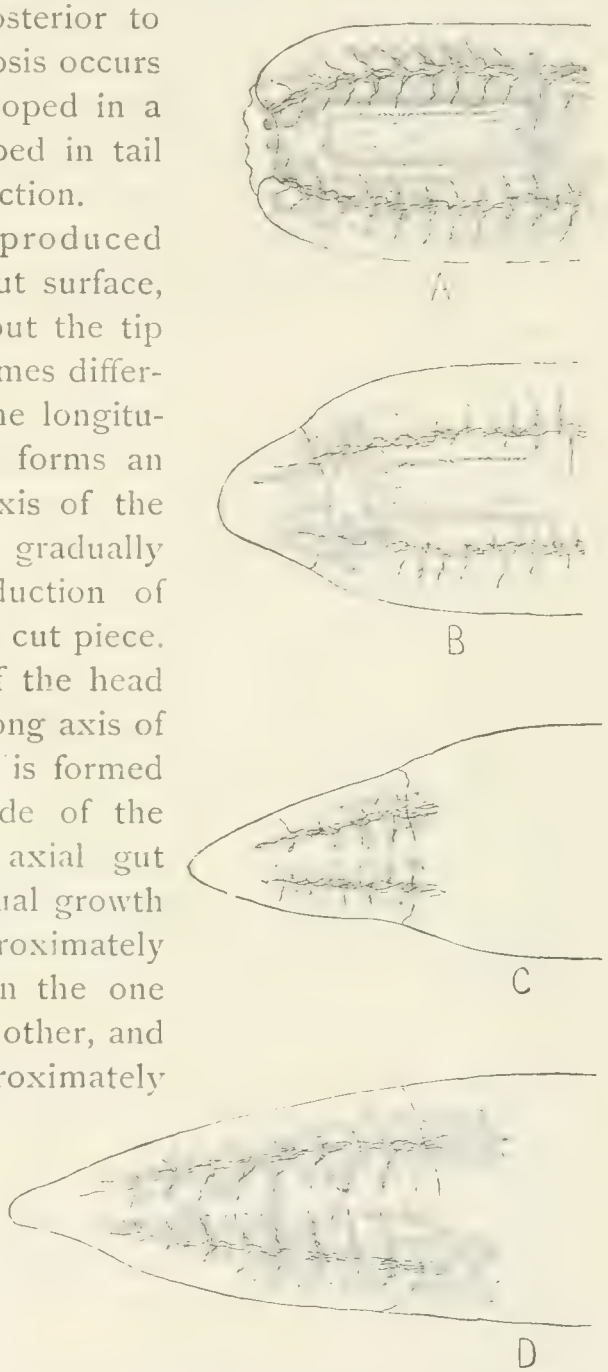


FIGURE 15. — To illustrate the regeneration of a new tail. A. Soon after section. B, C, D. Successive stages in the development of a new tail piece.

Regeneration in the front piece after transverse section posterior to the pharynx. — In the processes of regeneration of a normal worm from a tail piece, which we have just been considering,

that part of the tail piece posterior to the region where the new pharynx is formed remains essentially unchanged. The simplest phenomena seen in the regeneration of a new tail region may be followed at the posterior extremity of the worm from which the tail piece was severed. The regenerative processes after normal fission are essentially the same as after artificial transverse section.

The main steps of the process are as follows: —

1. Partial closure of the wound through the action of the musculature.

2. Protection of the cut surface, first by mucoid tissue, later by epithelium.

3. Multiplication of parenchyma cells near the cut surface. From the cell mass thus produced the musculature and parenchyma of the new tail are differentiated.

4. Posterior prolongation of the two main posterior trunks of the intestine. Branches may arise from these, and anastomosis between the latter often occurs.

5. Growth posteriorly of the two nerve cords and the innervation of new tissue by branches which arise from them.

6. Further differentiation into a tail of normal proportions.

The steps in this process are illustrated in Fig. 15.

1. If the section be made in the region indicated in Fig. 1 we find that the worm after a few spasmodic movements soon goes swimming or crawling unconcernedly about. As shown in Fig. 15, A, the musculature contracts the cut surface, though perhaps less vigorously than at the anterior end of the tail piece.

2. The protection of the cut surface is brought about in the same manner as at the anterior end of the tail piece, and therefore a description of the process may be omitted here.

3. The multiplication of cells at the cut surface is very vigorous, so vigorous that in from three to four weeks a new tail piece is produced of proportions similar to that of the lost tail.

4. As the new tissue develops the intestines grow back into it to supply it, much as blood vessels do in animals provided with a vascular system. The growth of the intestines takes place as described in the regeneration from the tail piece. The diagrams in Fig. 15 illustrate the process in general outlines.

5. The nerve cords grow backwards into the new tissue and occupy a position ventral and lateral to the two main posterior intestinal

branches. Growth takes place in a manner similar to that described in the regeneration of the nervous system in the tail piece.

6. The tail is gradually restored to normal proportions. In these sexually immature planarians the tail is a simple structure. It grows posteriorly until the pharynx comes to occupy a region midway between the anterior and posterior extremities of the worm. Normal intestinal balance is then set up and growth of the tail ceases.

Regeneration in the front piece after oblique section posterior to the pharynx. — The processes that take place here are essentially similar to those that take place after the tail has been severed by a transverse cut. Owing to the obliquity of the cut surface and the consequent contraction of the musculature, the long axis of the new tail is at first at an obtuse angle to the long axis of the worm. A greater growth, however, takes place on the short side of the animal so that the tail is gradually brought into line with the body. This greater growth on the short side may be due to the fact that the new tissue here gets a double intestinal supply (Fig. 16).

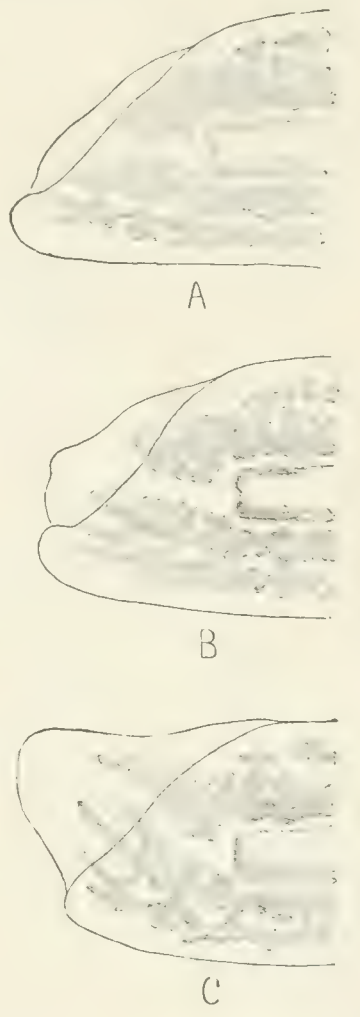


FIGURE 16. — Four stages in the development of a new tail after an oblique section posterior to the pharynx.

B. Regeneration after Dividing the Body in Various Ways.

Among the phenomena of regeneration which occur after severing a tail piece from the body may be recognized most of the essential features which occur in regeneration from pieces isolated from any region of the body. These essential phenomena may be grouped as follows:

1. Reduction of the surface exposed by the cut to the smallest possible dimensions. This is due to the action of the surrounding musculature.

2. Protection at the cut surface first by a transformation of all cells directly exposed to the water into mucoid cells and later by epithelium which extends outwards from the margins of the wound.

3. Multiplication of embryonic cells near the cut surface. From the cell mass thus produced the musculature and parenchyma of lost parts are differentiated according to the relation of the cell mass to the axial gut. Anterior to the axial gut the cell mass is differentiated into the tissue of the head, posterior to the axial gut into the tissue of the tail, and laterally to the axial gut into the tissue of the side of the body.

4. The axial gut may be formed, as in the case of the tail piece, by fusion or anastomosis of two or more branches of the intestine, or a single intestinal branch lying within the isolated piece may be transformed into an axial gut. From the axial gut branches extend into the newly formed tissue so as to supply it with nutriment just as newly formed tissue in animals having blood vessels is vascularized.

5. The tissue immediately posterior to the axial gut is transformed into embryonic tissue, and from this a pharynx and pharyngeal pocket are differentiated. Near the opening into the pharynx the axial gut gives off on each side one or more posterior intestinal branches. As a rule there is one principal trunk on each side of the body. These serve to supply nutriment to a newly developing tail. Posterior to the pharynx the tail develops until its tip is approximately the same distance as the anterior extremity from the pharyngeal pocket. The pharynx develops posterior to the point of least intestinal pressure, *i. e.* the point where intestinal contents are forced when the whole body is subject to spasmodic contraction.

6. The nervous system is developed in regenerated parts by outgrowth from the nervous system of the original isolated piece. The central nervous system is developed symmetrically with relation to the axial gut.

7. The isolated piece finally becomes differentiated into a worm of normal activities and proportions.

The reproductive organs are not regenerated in pieces isolated from animals sexually mature. It is probable, however, that they are developed later in the worms which are regenerated from isolated pieces just as they are from worms regenerated from pieces isolated from natural fission. For a brief note on this process see Curtis (1900).[1]

[1] Curtis: *Loc. cit.*

We shall now follow more specifically these essential phenomena of regeneration in the following classes of isolated pieces.

1. Head pieces.
2. Cross pieces.
3. Pieces containing pharyngeal and tail regions.
4. Side pieces.

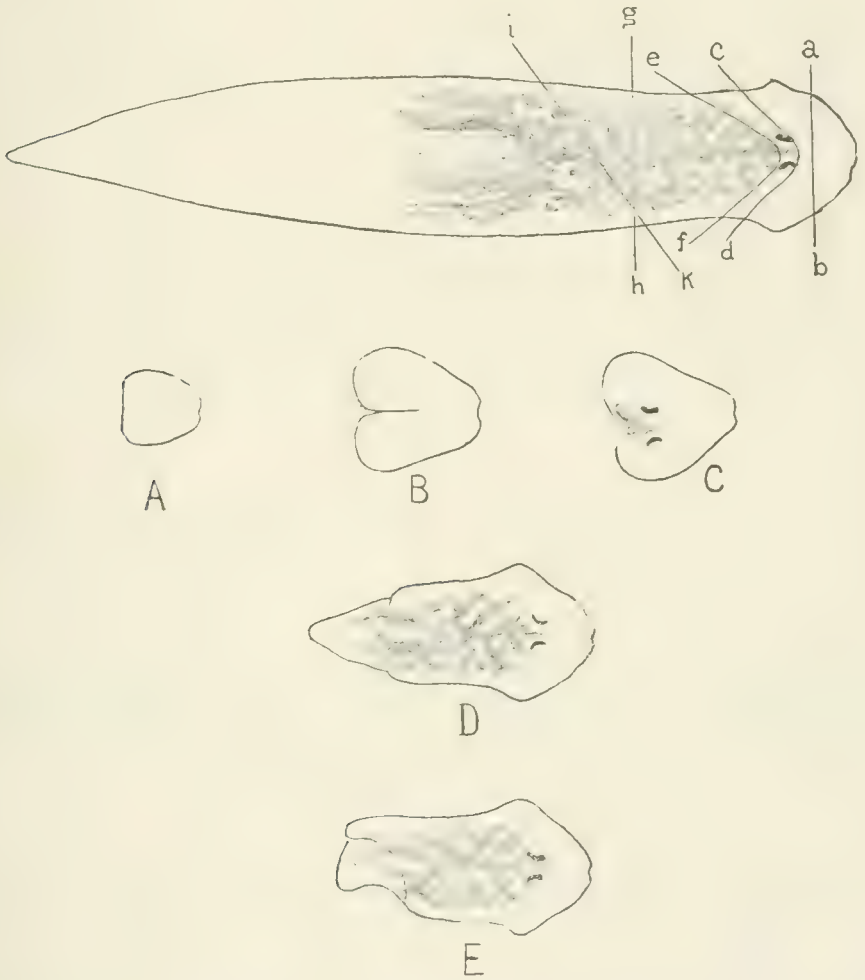


FIGURE 17. — To show the relation of the intestines to pieces cut from the worm anterior to the pharynx. The pieces are represented as they appear 3–4 days after the operation. A. Piece cut by the incision a–b. B. Piece cut by the incision c–d. A and B contain no intestinal tissue. C. Piece cut by the incision e–f. The small tips of intestine which the piece removed contained have fused into a single axial gut. D. Head piece isolated by simple transverse section (g–h). E. Head piece isolated by oblique section (i–k).

Regeneration in head pieces. — If a transverse or oblique section be made through the body of a planarian between the region of the auricular appendages and the pharynx, the part of the body thus removed, including the pharynx, is readily regenerated. Morgan has shown that a piece isolated by making a cut anterior to the eyes

(a–b, Fig. 17) will not regenerate. I have found that the regeneration of a head piece depends on whether or not some of the intestine is contained in the piece isolated. The principal phenomena noted when section is made through the body anterior to the pharynx are as follows:

If the cut be made anterior to the eyes (a–b, Fig. 18) the piece isolated may live for three days or more before disintegrating. During this time the exposed surface becomes covered with epithelium and the piece may move about. No further regeneration than that of the superficial epithelium occurred in any of the pieces with which I experimented. What might happen could they be kept alive longer I do not know. My pieces seemed to succumb to bacteria.

If a circular cut be made just anterior to the intestines so that no intestinal tissue is included in the piece isolated (c–d, Fig. 17), the cut surfaces will heal together (Fig. 17, B). I have been able to keep such a piece alive five to six days. No internal differentiation was noted.

If a curved section be made slightly posterior to the one just mentioned, so as to include the tips of the anterior intestinal branches (e–f, Fig. 17) the cut edges will heal together (C, Fig. 17) and the bits of intestine will fuse to form a single axial gut. Such a piece I have kept alive for ten days. No pharynx had formed during this period, but the density of tissue at the posterior end of the gut seemed to indicate that a pharynx was about to form. Unfortunately the piece that lived longest died during the night, so that the tissue was not in condition for microscopic examination.

If a transverse cut be made between the region of the auricular appendages and the pharynx, the cut surface is protected at first by the action of the musculature, by the exposed surface cells and the epithelium, in the manner previously described for similar conditions. A mass of embryonic cells quickly collects at the posterior extremity of the head piece. About the posterior extremity of the axial gut embryonic tissue is formed from which the pharynx and the pharyngeal pocket are differentiated. Under favorable conditions the pharynx may be differentiated in two days. This rapidity is due in the main to the fact that the process of retrograde metamorphosis necessary before a pharynx can be developed in a tail piece is here unnecessary since the embryonic tissue of the cut surface is utilized.

From the lateral branches nearest to the new pharynx are developed the main posterior intestinal trunks. These extend back into

the tissue posterior to the pharynx, which rapidly assumes the characteristics of a tail. The nerve cords grow posteriorly, lateral and ventral to the main posterior intestinal trunks.

If the head piece is separated by an oblique cut the phenomena are essentially the same, except that the tail develops at first at an obtuse angle, and only later is brought into the axial line (Fig. 17, E).

Regeneration in cross pieces. — Cross pieces develop anteriorly a head region, posteriorly a tail region, and posterior to the point of least intestinal pressure a new pharynx. They always contract laterally and expand in length during this process.

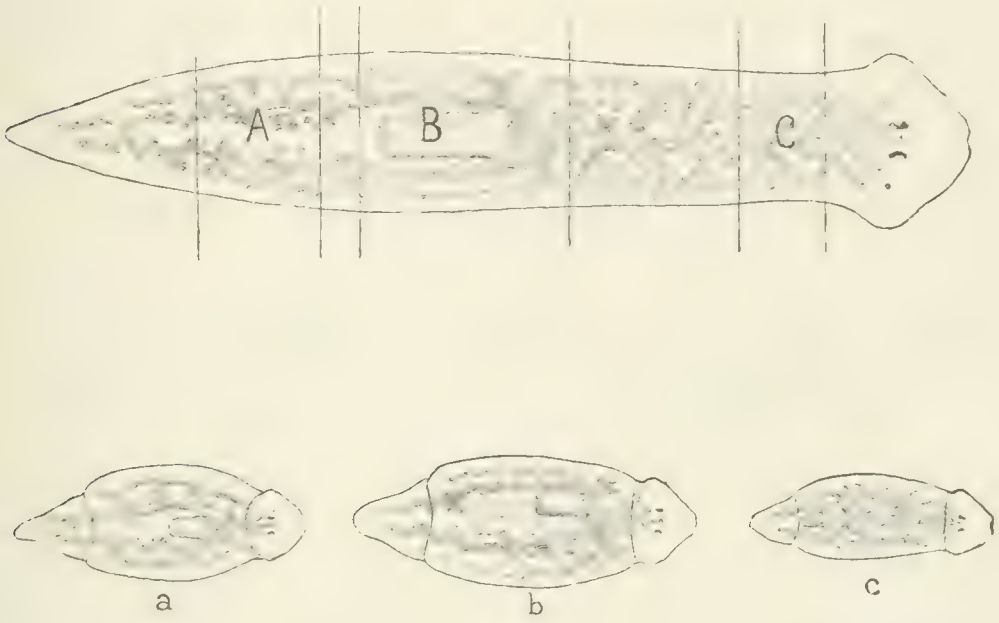


FIGURE 18. — Showing the regions from which transverse pieces were cut, and the worms regenerated from each of these regions about ten days after dividing the animal. A, a. Transverse piece from a region posterior to the pharynx. B, b. Transverse piece through the region of the pharynx. C, c. Transverse piece through the region anterior to the pharynx. The developed worms are a little too large in proportion to the figure of the parent worm.

a. Transverse cross pieces. — If the cross pieces are severed from the body by transverse section the phenomena of regeneration are very simple (Fig. 18). Such pieces may be taken from the region posterior to the pharynx, from the pharyngeal region and from the region anterior to the pharynx.

1. In case the cross piece is taken from the region posterior to the pharynx, the head is developed in a manner similar to that described for tail pieces. The regeneration is not quite so rapid as in the case of a tail piece. The new tail is regenerated in the manner

described as occurring when the region posterior to the pharynx is removed from a normal worm.

2. If the cross piece is taken from the pharyngeal region, the pharynx lends complexity to the situation.

As a rule, if the cross piece is confined to the pharyngeal region, the pharynx becomes united by a growth of tissue from the walls of the pharyngeal pocket to the latter, retrograde metamorphosis sets in, and the pharynx is finally replaced by normal parenchyma. Meanwhile another pharynx is developed posterior to the point of least intestinal pressure. The reason for this replacement of the old pharynx by a new one is probably due to the lack of support offered by the cross piece to the old pharynx in its movements (Fig. 18, B). From a pharynx alone a new worm is never developed.

If the cut separating the cross piece anteriorly passes close to the base of the pharynx it usually takes some time for a head to develop, owing to the obstacles set in the way of the development of an axial gut. Thus I have seen cross pieces cut anteriorly and posteriorly to the pharyngeal cross piece develop heads with eyes several days before the pharyngeal piece. If the cut passes a slight distance in front of the base of the pharynx (Fig. 18, B) so that the cross piece has an axial gut and two main posterior intestinal trunks, a new head piece is very rapidly regenerated symmetrically in front of the axial gut. A new tail is regenerated from the cross piece in the manner described for the development of a new tail posterior to the pharynx.

3. A cross piece cut anterior to the pharynx very readily regenerates both head, tail, and pharynx. The axial gut of the parent worm becomes the axial gut of the new worm. The new head is developed symmetrically in front of the axial gut. The new pharynx is developed posterior to the point of least intestinal pressure, which here corresponds closely to the end of the axial gut. The new tail is developed symmetrically posterior to the pharynx, and branches from the axial gut extend back into it to form the posterior intestinal trunks (Fig. 18, C). As in the case of head pieces the pharynx develops rapidly in from two to three days.

b. Oblique cross pieces.—The phenomena seen in oblique cross pieces are very similar in nature to those seen in transverse cross pieces, though some slight differences prevail. The phenomena occurring in oblique cross pieces through the region posterior to the pharynx are similar to those seen in the regenerating tail piece and body after the tail has been severed by an oblique cut from the

body. The phenomena occurring at the anterior end of an oblique section through the region of the pharynx are similar to those occurring at the anterior end of an oblique cross piece from the region in front of the pharynx.

The processes occurring in the latter are shown diagrammatically in Fig. 19. The pharynx seems to develop always at the posterior extremity of the old axial gut. On the other hand, as shown in Fig 19, in case of a very oblique cross piece a lateral branch of the old axial gut may be transformed into an extension of the axial gut. The head then develops symmetrically about the tip of this, and hence somewhat lateral to the axis of the parent worm. The new worms are much narrower and longer than the transverse pieces from which they develop (Fig. 1).

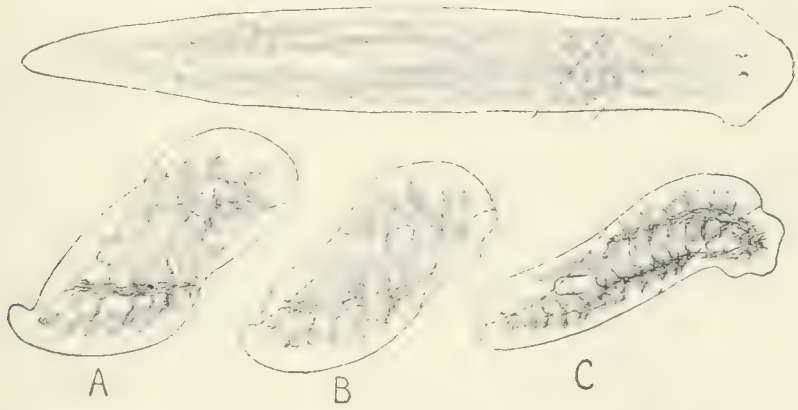


FIGURE 19. — Development of an oblique cross piece taken from in front of the pharynx. A. 24 hours after removal. B. 48 hours after removal. C. 96 hours after removal.

Regeneration in pieces containing pharyngeal and tail regions. — If the body of a worm is cut transversely or obliquely anterior to the region of the pharynx, the piece posterior to the cut will restore the lost parts. If the cut is made near the base of the pharynx a new pharynx may be developed. The phenomena of regeneration of the lost parts are essentially similar to those described as occurring in the regeneration of the head area in cross pieces, so that a specific description of them is not necessary here. A new head area seems to be regenerated almost as quickly on the anterior cut surface of a cross piece as on the anterior cut surface of a piece not deprived of pharyngeal and tail regions. To determine the influence of the latter, I performed the following experiment:

Fifteen worms were decapitated by the cut a-b, Fig. 20. On the following day an anterior cross piece, a b d c, was removed from five

of these worms, and on the third day after the operation a similar cross piece was removed from five more. At the close of the third day, therefore, I had —

Lot A. 5 pieces, e a b.
Lot B. 5 pieces deprived of e c d for 2 days.
Lot C. 5 pieces deprived of e c d for a few hours.
10 pieces e c d, which can be disregarded.

In all three lots, A, B, and C, the head areas began to be well differentiated by the fourth day after the original operation. In individual pieces in each of the three lots eye spots could be seen, and on the fifth day these were visible in the heads of all the individuals in each lot. The deprivation of œsophagus and tail regions seems therefore to exercise no deleterious effect upon the development of a new head region.

Regeneration of lateral areas and in side pieces. — If an area lateral to the axial gut be removed from a worm this area is usually replaced without essential morphological alteration in the rest of the worm.

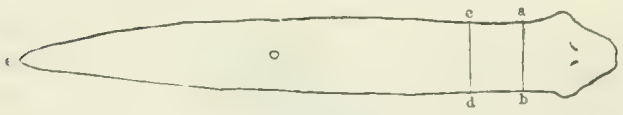


FIGURE 20. — (See page 35.)

From a lateral area (side piece) thus removed, if it is not too small, a new worm will be regenerated of smaller size than the original worm.

a. Restoration of lost lateral areas. — If a longitudinal lateral area is removed, as after making the cut a-b, Fig. 21, the worm contracts at once on the side towards the cut surface, thus reducing this to the smallest limits. The nearer the middle line the cut comes, the greater is the contraction. Along this cut area embryonic tissue is formed and this is gradually differentiated, so that the area to the right of the mid-line of the animal becomes similar to that to the left of the mid-line. This transformation of embryonic tissue is due in all probability to the fact that on the cut side the intestinal branches are short and intestinal fluids are sent with especial force into the tissue on that side of the worm. Growth continues until the intestinal balance is restored. The development of tissue on the cut side gradually forces the worm back into a position in which its long axis forms a straight line. New intestinal branches grow out into the new tissue. If the cut has been made near enough the mid-line to remove a nerve cord, the latter is restored from that part of the central nervous system which remains. From the brain there is a

lateral growth of nervous tissue which restores the lost part. While this is being restored a new nerve cord grows tailwards from the region of the brain. This growth takes place in the newly formed tissue. The lower end of the cord may be restored from what remains of the original cord, as is shown in Fig. 21, A. If starved, the animal as a whole becomes smaller during this process.

If a lateral area is removed from the region of the head (c e d, Fig. 21), the remaining half of the head will be contracted towards the cut and may be made to become attached to the anterior cut surface

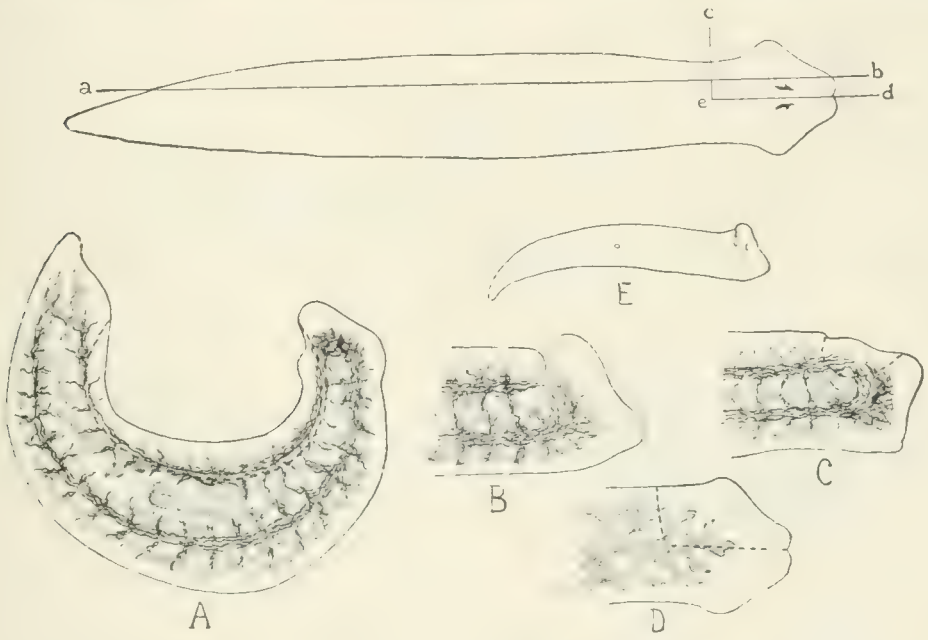


FIGURE 21. — To illustrate the restoration of lost lateral areas. A. Contraction of the worm towards the cut surface, 3 days after making the cut a–b. B. Contraction of head after removing the piece c e d. C — D. Gradual restoration of head to normal position. E. Showing the bending of the tail to the opposite side after removing the piece c e d.

(B, Fig. 21). For a time the auricular appendage may then act the part of a "head," or advance feeler, as the worm wanders about. It does not, however, become converted into a head. On the contrary, the lost tissue is gradually restored (Fig. 21, C and D), until the head once more becomes symmetrically arranged about the anterior extremity of the axial gut. The whole process of restoration takes about a week. It is of interest to note that the tail often bends towards the side opposite the cut after this operation (Fig. 21, E).

If the pharynx is destroyed by the removal of a lateral piece, it is quickly replaced by the development of a new one posterior to the region of least intestinal pressure.

These two examples may suffice to indicate, in a general way, the nature of the process of the restoration of parts lost from the side of the body.

b. Regeneration of a normal worm from a side piece.— Side pieces may be removed from the body by a great variety of cuts. In all cases subsequent development is essentially similar.

1. Owing to the contraction of the longitudinal musculature along the exposed surface, a side piece is quickly curled into circular form (Fig. 22, A).

2. The violent contraction of the musculature forces out a considerable amount of parenchyma tissue and this soon partially fills in

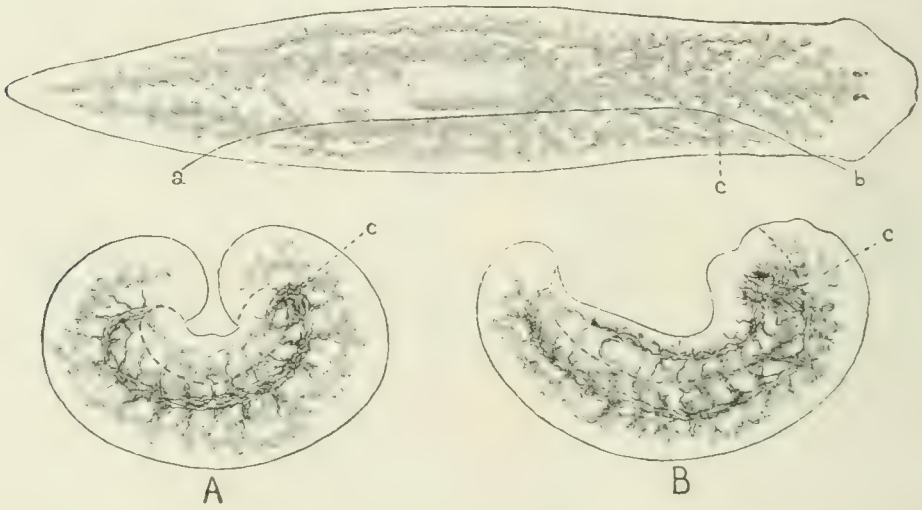


FIGURE 22.—To illustrate the regeneration of a worm from a side piece. a–b. Line of section. c. Point opposite which head develops. A. Two days after removal. B. One week after removal.

the centre of the ring-shaped body formed by the contraction of the musculature (Fig. 22, A). This shortly gives place to an embryonic cell mass, which is gradually differentiated into head, tail, and lateral regions of the new worm.

3. The axial gut is formed usually by anastomotic branches which serve to join together the cut ends of the branches of the original axial gut (Fig. 22, A). Soon after the axial gut is formed a new pharynx is differentiated posterior to the point of least intestinal pressure. In the piece illustrated in Fig. 22, B, this took from six to seven days. The head is developed symmetrically about the anterior end of the newly formed axial gut.[1]

[1] MORGAN (*loc. cit.*) has shown that small side strips may develop a head, in one instance two heads, the axis of which lies at right angles to the original long

The nervous system develops from the lateral cord (A and B, Fig. 22). The brain is developed at the anterior end of the lateral cord, and the lateral cord for the opposite side of the body then develops posteriorly in the new tissue. When the side piece is a narrow one the lateral cord may lie for a time directly ventral to the axial gut and give rise to lateral branches for the innervation of the new worm on each side. Whether such a condition may remain permanent, or whether eventually the cord always becomes shifted relative to the axial gut and a new one is developed on the regenerated side, I have not determined.

In considerable measure the development of the eyes depends on the development of the lateral cords. As a rule in these side pieces the eye that develops on the side of the body on which the lateral cord has remained reaches maturity much earlier than the one on the side where the cord is restored.

I have been unsuccessful in my attempts to keep alive side pieces cut lateral to the nerve cords, and so I am unable to state whether a new nervous system can be developed if the old central nervous system is entirely removed.

In the further process of development the new tissue gradually comes to exceed the old in amount, so that the old tissue finally occupies a position in the same worm relatively the same as in the original piece (Fig. 22, b).

Small side pieces developed much less rapidly than larger ones.

C. Regeneration of Pieces Incompletely Separated from the Body, and the Production of Freaks.

The phenomena of regeneration which we have hitherto discussed, whereby lost parts are restored and small pieces of a worm are developed into normal worms, differ in no way essentially from the phenomena which occur when monsters with double heads or double tails, and other freaks are produced after cutting a worm. On the other hand, this production of freaks offers perhaps the best means of gaining insight into the phenomena of regeneration.

A consideration of the cases hitherto described will show that the following conditions are necessary for the production of a head region, a tail region, a pharynx, and a lateral region.

axis of the body. In the one instance in which I was successful in causing a head to arise in this manner the piece was destroyed before I could follow the essential internal features of the process.

1. A head is produced when an anteriorly directed main intestinal trunk (axial gut) is directed against newly formed embryonic tissue. The head is then formed radially about the end of the gut.

2. A tail is produced from embryonic tissue about the end of main intestinal branches posteriorly directed and lying posterior to the point of least intestinal pressure.

3. A pharynx is formed just posterior to the point of least intestinal pressure. If normal parenchyma lies here it is converted first into embryonic tissue, and from the latter the pharynx and pharyngeal pocket are differentiated.

4. A lateral region is developed from embryonic tissue lying at

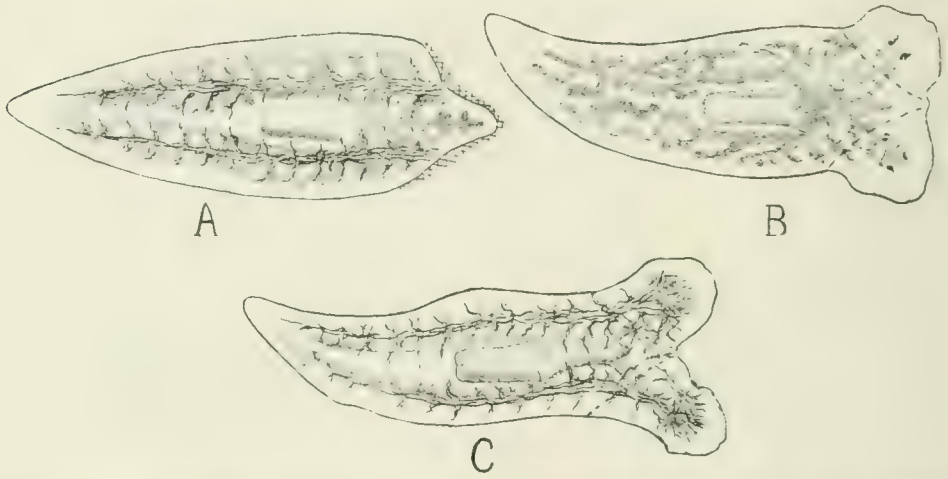


FIGURE 23. — To illustrate the development of two heads when two oblique surfaces are exposed anteriorly. A. Condition 2 hours after making cut. B. Condition 4 days after making cut. The nervous system is not represented. C. Condition 18 days after making cut. The intestinal system is represented very diagrammatically. In reality the branchings at this stage resembled closely those shown in B.

one side of the main intestinal trunks. Development continues until the newly formed tissue is sufficient to counterbalance in amount the original tissue lying on the opposite side of the gut, or until the normal proportions of the worm are reached.

We shall consider in this section the production (1) of two or more head regions and (2) of two or more tail regions, taking up, in considering each of these, the regeneration of new pharynxes and lateral regions.

I. The regeneration of two or more head regions. — a. New heads directed away from axial line. — If the body is cut as indicated in Fig. 23, either in front of or behind the pharynx, as a rule two heads develop, see Fig. 23, A, B, and C. This is due to the fact that much more embryonic tissue is produced in front of each oblique cut

surface than in front of the angle at which they meet anteriorly. The main lateral branch on each side of the axial gut grows forward into this embryonic tissue, and thus these lateral branches soon exceed in length and importance that part of the original axial gut lying anterior to the point where the lateral branches are given off. The retrograde process in the axial gut is furthered by the injury naturally done it in making the section. Each lateral branch takes on the functions of an axial gut, and we have two new axial guts, uniting in front of the pharynx if the cut is made as represented in Fig. 23. About the extremities of these axial guts new head regions are formed in the usual manner.

The growth of the central nervous system is interesting. A well marked commissure is developed in the region just posterior to



FIGURE 24. — To show the development of a head in tissue posteriorly directed. A. Soon after section, ventral view. B. One week after section, ventral view. C. Three weeks after section, dorsal view.

where the two head regions meet in the middle line (Fig. 23, C.) This commissure is intimately related to the brain of each new head and takes the place of a lateral cord.

Occasionally a head may be developed from embryonic tissue produced on a cut surface which is posterior in respect to the musculature bordering on this surface. Van Duyne gave an illustration of such an instance as a proof of heteromorphosis. The most interesting specimen of the kind which I have had the opportunity of studying is shown in Fig. 24.

In this instance, in an oblique cross piece a lateral intestinal branch sent a large process posteriorly and another anteriorly (Fig. 24, A). About the tips of both posterior and anterior intestinal processes new heads developed. Here a lateral branch of the original axial gut became a bi-axial gut of the regenerating worm.

Stimulation of either head caused movement in the opposite head before the body was set in motion. Each head piece became capable of extensive movements in various directions. For the most part the movements of one head were independent of those of the other head. The worm was accidentally destroyed, so that I could not study its nervous system.

In spite of repeated trials I was unable to find another worm in which the intestines were so arranged that a cross section like the above resulted in producing a similar double-headed monster.

b. New heads directed towards the axial line. — A new head is usually developed at the anterior end of the median surface of a piece partially isolated by making a posteriorly directed longitudinal incision. A head is often developed at the anterior end of the cut surface when the incision is directed anteriorly.

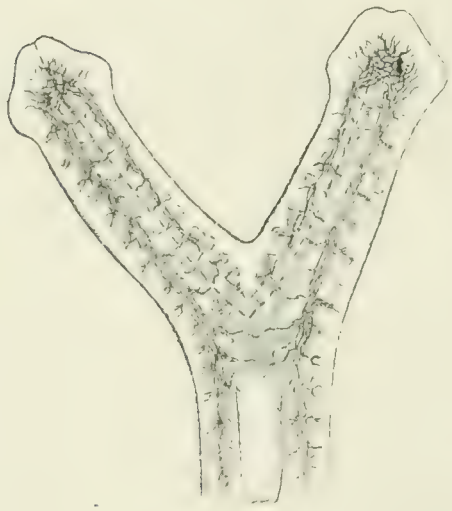


FIGURE 25. — To illustrate the regeneration after a median longitudinal incision in front of the pharynx.

1. *New heads after a longitudinal incision directed posteriorly. a. When the incision is made anterior to the pharynx.*

If a longitudinal incision is made in the median line from the tip of the head towards the pharynx, and the incision is kept open, each half-head piece will regenerate a new head, so that we have a double-headed monster.

Immediately after the incision the two head pieces contract so as to curl inwards across the median line. By this muscular activity not only is the area of each cut surface reduced, but also the cut surfaces are brought into close contact near the limit of the incision. At this point suturing begins, and often will extend rapidly headwards, so that the two head pieces will be once more united unless this is artificially prevented.

If this is prevented a new axial gut is developed near the cut surface in each head piece. These axial guts are developed in part from the remains of the original axial gut if the incision has not been quite in the median line, and in part from anastomoses which are formed between the cut ends of the lateral branches of the original axial gut.

As new embryonic tissue is produced along the cut surface, lateral branches are sent out into this from the axial gut. By the time the area of the newly formed tissue equals in extent that of the original tissue lying on the opposite side of the axial gut, and bilateral symmetry has been restored, the worm when flattened presents the appearance seen in Fig. 25. The two heads may have very independent movement. The nervous system is regenerated in the manner previously described for side pieces. In one instance no new lateral cord developed on the side towards the cut surface of one of the head pieces, and on this side only one eye developed.

If the incision is made lateral to the median line (Fig. 26) the cut ends of the lateral branches of the axial gut become united by anastomotic branches, a new axial gut is formed, and a head piece becomes symmetrically arranged about this. The remarks applied to the development of the nervous system in side pieces will apply here also. Occasionally a new lateral cord is not developed, and the slip has a head with but one eye.



FIGURE 26. — To illustrate the regeneration after a lateral longitudinal incision in front of the pharynx.

Usually in these double-headed monsters the pharynx becomes considerably hypertrophied.

b. When the incision is made posterior to the pharynx. — If the incision passes near enough the median line to include one of the two main posterior intestinal trunks, this becomes the axial gut of the attached side piece. Otherwise some of the lateral branches subserve this function. The experiment succeeds much better when the main intestinal trunk is included in the side piece. In Fig. 27 is given an illustration of an instance in which a lateral branch has become converted into the axial gut of the side piece. As in all other cases, the new head and side develop symmetrically about the new axial gut. The new nervous system is developed from that part of the lateral cord which is included in the side piece. A new pharynx was developed in all my specimens in which a new head was developed from a side slip. The pharynx was in all instances developed posterior to the point of least intestinal pressure. (See Fig. 27.) Lemon

pictures a pharynx in most of the specimens on which he tried a similar experiment.

In a certain number of instances no head develops about a slip separated by a posterior longitudinal incision in this region. Such an instance is shown in Fig. 28. The new tissue is so placed about the axial gut as to give bilateral symmetry to the slip, but no head has been differentiated about the extremity of the slip and no pharynx has been formed. It has seemed to me after a careful study of a number of similar instances that the explanation lies in the fact that the movements of the musculature of the slips in these instances are controlled by the nervous system of the parent worm, and not by an independent nervous system. Such slips at no time show well marked automatic power. A definite action of the body musculature is necessary to set up nutritive currents of sufficient intensity to cause the differentiation of head tissue.

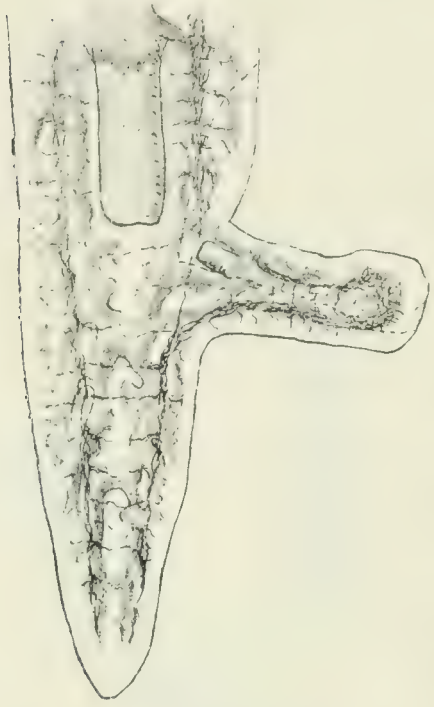


FIGURE 27. — To illustrate the regeneration after a longitudinal incision posterior to the pharynx. Nine days after making the incision.



FIGURE 28. — To illustrate an instance where a head failed to develop at the extremity of a slip separated by an incision directed posteriorly.

2. *New heads after a longitudinal incision directed anteriorly.* — A new head will be regenerated on the anterior end of a cut surface after a longitudinal incision directed anteriorly, provided a main intestinal trunk is severed so that the gut terminates in this region. This is likely to occur if the incision is made in the median line and carried well towards the head.

Fig. 29 shows the conditions which give rise to two heads in this situation. The longitudinal slip is made as shown in A. The effect

of this incision is to destroy the original axial gut except in the region of the head (Fig. 29, B). The lateral branches of the axial gut become united near the cut surface by anastomosis, but the anastomosis does not extend to the axial gut of the head. Each newly formed axial gut, therefore, terminates near a fresh cut surface (Fig. 29, B) and a new head becomes formed symmetrically about this terminal axial gut (Fig. 29, C). A new pharynx is formed on each side at the place of least intestinal pressure (Fig. 29, C). Nutrition is conveyed into the old head by peripheral anastomotic branches.

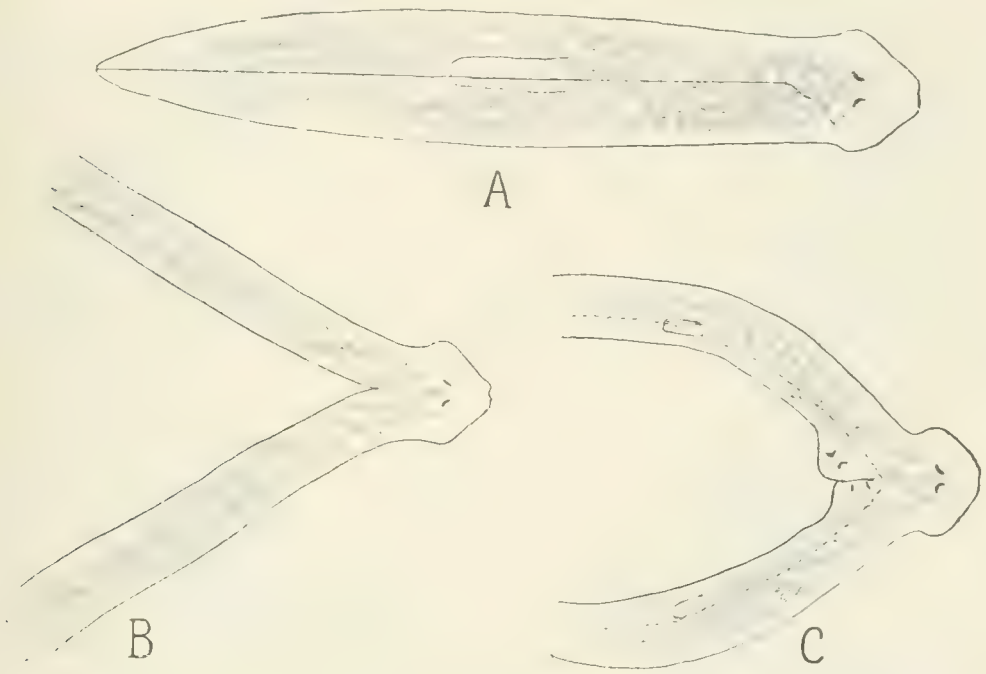


FIGURE 29.—To illustrate the conditions which give rise to the development of heads at the anterior end of a longitudinal slit directed anteriorly. A. Showing region of cut. B. Conditions immediately after cut. C. One week after cut.

If large anastomotic branches remain or are developed between the new axial guts and the intestines of the head, no new head is formed (Fig. 30).

If the original axial gut is left on one side (Fig. 31) no new head is developed on that side. A head may be developed on the opposite side. In Fig. 31 an instance of this kind is pictured. In this case, however, a portion of a head was developed on the side where the axial gut was continued nearly uninterrupted into the region of the head. The partial development of the head on the left side was due to a lack of complete union between the axial gut of the side and of the head.

This specimen was also interesting because illustrating a not uncommon sight, two pharynxes evidently representing two points of

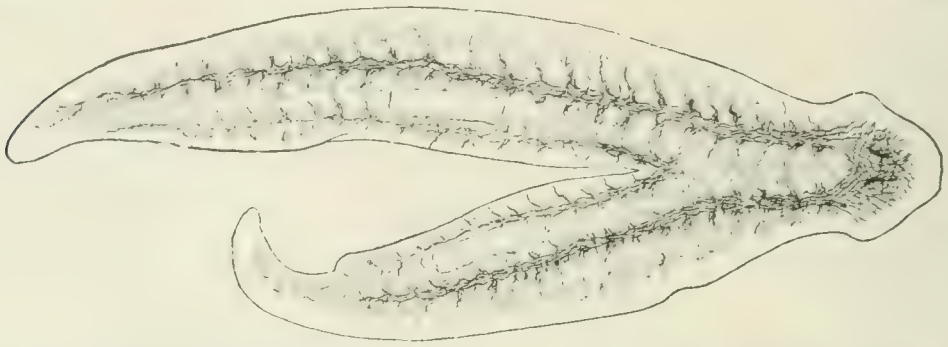


FIGURE 30. — To show the arrangement of the intestines and nervous system after a longitudinal slit in which no new heads developed.

least intestinal pressure on the right side. (The specimen is pictured from the ventral surface.)

If the median incision is carried to or beyond the eyes, each half head is restored by the outgrowth and differentiation of embryonic tissue (Fig. 32).

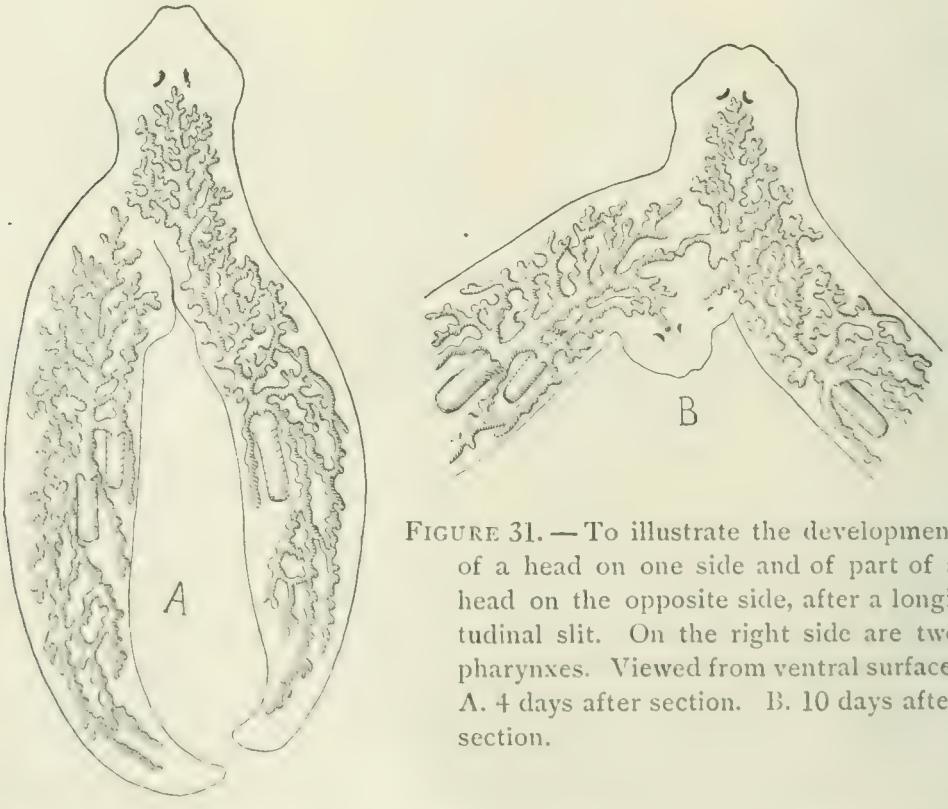


FIGURE 31. — To illustrate the development of a head on one side and of part of a head on the opposite side, after a longitudinal slit. On the right side are two pharynxes. Viewed from ventral surface. A. 4 days after section. B. 10 days after section.

In all the instances mentioned in this section bilateral symmetry is restored in respect to the axis extending through the new pharynx to the centre of the new head.

II. Regeneration of two or more tail regions. — Tail-like processes are symmetrically formed about the included intestinal branches whenever a slip is partially separated from the body by a slit extending in

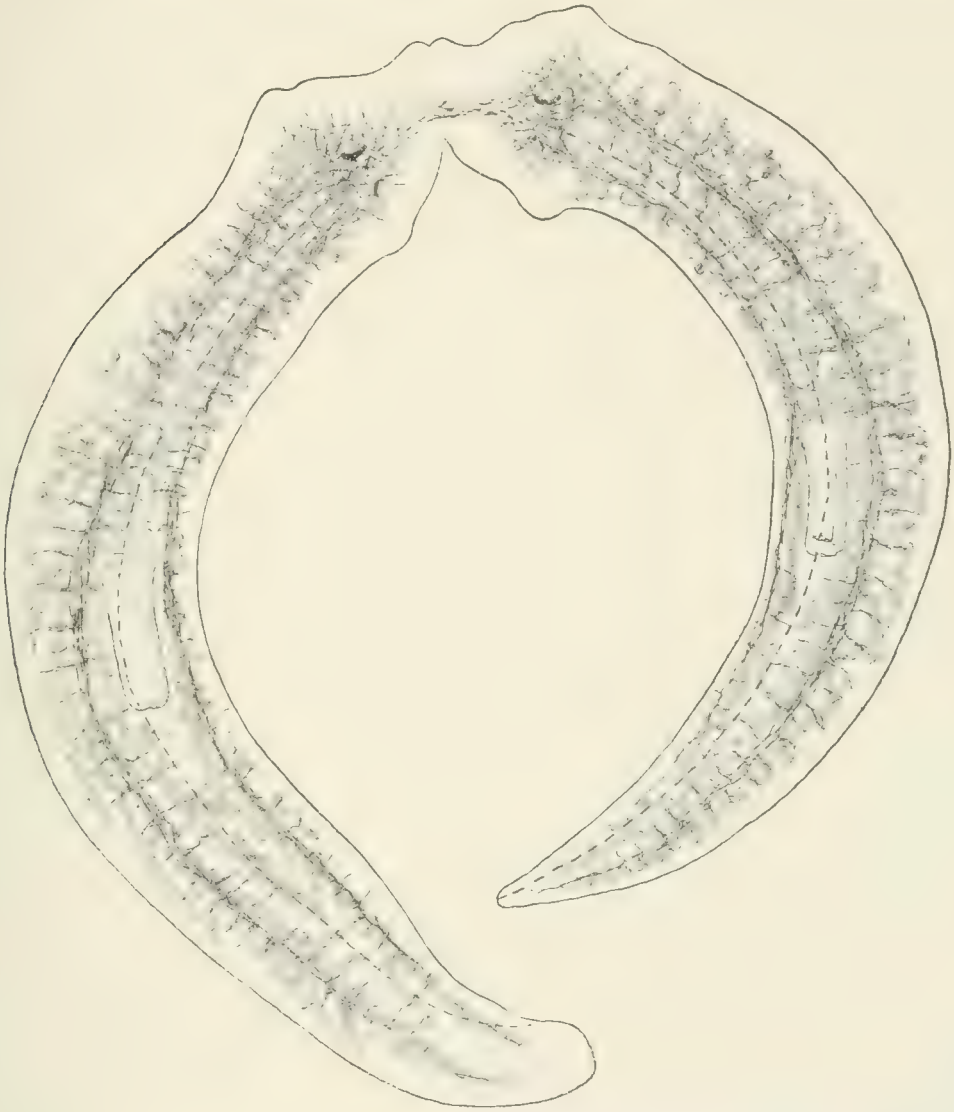


FIGURE 32. — Showing the parts restored after a longitudinal incision extending from the tip of the tail to the region of the eyes.

an anterior direction. These tail-like processes execute movements which correspond in nature and time with those of the main tail region, if this remains. If the slit extends far anteriorly a new pharynx will be formed in the tail piece (Fig. 30).

V. SUMMARY AND THEORETICAL CONCLUSIONS.

The facts recorded in the foregoing pages refer to phenomena the truth of which can be readily verified in a few weeks by one who has

at hand an abundance of planarian material. The phenomena shown by the intestines during regeneration may easily be followed from day to day in small, thin, slightly pigmented worms, especially if a piece of cover-glass is used to exert pressure upon them. The regenerating pieces live well if placed in small shallow dishes kept very clean. In most instances I kept my worms in rain water. This probably contains some food material for them, but they decrease in size unless fed. For the latter purpose crushed fresh-water snails are exceptionally good, a method suggested to me by Winterton Curtis.

Studies of the regenerative changes in the nervous system are far less satisfactory than those of the intestines because they cannot be followed in the same specimen from day to day. Instead, similar pieces must be hardened, sectioned and stained at different stages. For general work a dilute Zenker's Fluid makes a good fixing agent. Iron-hæmatoxylin and dilute hæmatoxylin followed by Congo red make satisfactory stains. Only the coarser general features of the nervous system, however, can be followed by this method. It is important that special selective stains be used in this study. This I have not done.

The diagrammatic drawings accompanying this paper have been based so far as possible on actual specimens, being, except in case of the nervous system, in the main, free hand sketches made from the living specimens. No attempt has been made to preserve accurately the relative size of the various specimens at different stages.

For a summary of the results obtained the reader is referred to the headings of the various sections at pages 19, 29, and 40.

In this place I desire to discuss merely the most general results and the conclusions I have drawn from them.

Regeneration takes place by the transformation of adult tissue into embryonic tissue, and the differentiation of the latter into parts and organs which restore to a small or large sized piece of a worm those parts which are lacking to make it a complete individual.

I. Embryonic tissue is formed in the specimens here studied in two places only, (1) at or near a cut surface and (2) in the region of the piece just posterior to the point of least intestinal pressure. By embryonic tissue is meant a tissue composed of small ovoid closely packed cells with large nuclei.

The causes of the production of embryonic tissue at or near the cut surface are hypothetical. It may be that a slight change in osmotic pressure due to the exposure of internal tissues to the water is

responsible. It may be that enzymes are set free by the injury (Loeb, 1900).[1] I have tried cutting planarians in two with a red-hot knife to see if cauterization would prevent the formation of tissue. Owing to the small size of the Planaria maculata and to the moist medium with which it is surrounded even when removed from the water, too much injury is done by the heat to the whole worm, to make the experiment a success. It would be well to try the experiment on larger planarians, on a land planarian for instance. Whatever may be the cause, however, the embryonic tissue is always formed at and near a cut surface.

The cause of the formation of embryonic tissue just posterior to the point of least intestinal pressure is equally dark. The process is much slower and is preceded by a retrograde metamorphosis in the pre-existing adult tissue. We might assume that here certain intestinal fluids are set free by pressure.

II. The differentiation of this embryonic tissue depends on its relations to the intestinal apparatus of the animal. If it lies anterior to the main axial gut it becomes converted symmetrically into a head region. If part of the old head remains in this situation the new material serves to supply the missing parts of the head.

If the embryonic tissue lies lateral to the axial gut or to a line extending directly posterior to this it becomes converted into a new lateral region of the body, so that the worm becomes bilaterally symmetrical in respect to the axial gut.

If the embryonic tissue lies at the posterior end of the axial gut, *i.e.* behind the point of least intestinal pressure, it becomes converted into a new pharynx and pharyngeal pocket.

If the embryonic tissue lies posterior to the pharyngeal region it becomes converted into a new tail. This tail region is supplied by branches which extend from the axial gut around the pharyngeal pocket into it, and it becomes symmetrically arranged in relation to these main posterior intestinal branches.

So far as is compatible with the regeneration of a whole worm from a part of a worm, the piece from which the new worm is restored occupies essentially the same position in the new worm that it did in the old, and remains in its internal structure essentially unaltered.

Unlike the pharynx, neither the head nor the tail areas can be formed unless embryonic tissue is produced by exposure of a cut

[1] Loeb: This journal, 1900, iv, p. 60.

or torn surface. Thus in Fig. 21 is shown an instance in which the auricular appendage occupied a position anterior to a main intestinal branch, yet the tissue of the auricular appendage did not become converted into head tissue. On the contrary, the lost part of the head was restored by a growth of new tissue in the region of the cut surface.

I also tried the experiment of getting two lateral slips to heal together before the embryonic tissue of the exposed surface could be specifically differentiated. Cuts were made as shown in Fig. 33, A, and the cut surfaces were made to heal together as shown in Fig. 33, B. In most of the instances in which I tried this experiment the healing together proved to be incomplete at the anterior end of the worm, and from the mass of embryonic tissue here exposed a new head was very slowly formed. In one instance no head developed. The worm died after I had it under observation for about ten days so that I do not know whether or not a head might subsequently have developed.

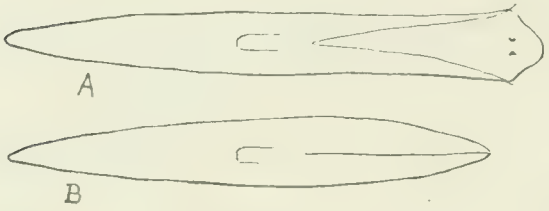


FIGURE 33. — To illustrate an attempt made to prevent the formation of a new head at the anterior end of a piece. A. Showing the lines of the cut. B. Showing conditions after cut surfaces united.

III. In this article I have taken up the study of regeneration almost wholly from the point of view of the relations of the newly developing parts to the intestines and especially to the axial gut. We have now to inquire a little more specifically into the nature of this relation. Has the intestinal system a specific action in determining the nature of the parts developed from the embryonic tissue or is the development of the intestinal system merely a coincident phenomenon? It has seemed to me that the intestinal system is the means through which a specific action is exerted on the embryonic tissue from which the lost parts are differentiated.

As we have seen in the various experiments explained in this article, the first step in the specific differentiation of parts in the regeneration of a new worm from a piece of worm is the formation or selection of an axial gut. If part of the old intestinal system is so situated that it can be utilized for this purpose it is at once converted into an axial gut. Otherwise, the axial gut is formed by fusion and anastomosis between the branches of the original intestinal system.

When the axial gut is formed we find a head differentiated about its anterior extremity, a pharynx about its posterior extremity, and lateral areas on the side towards the cut surface. This differentiation we assume to be due to nutritional currents of a specific direction, intensity, and force. Analogy is dangerous, yet within limits we can compare the action of the axial gut upon embryonic tissue to the action of a bar magnet upon iron filings scattered upon a paper placed above it. In the case of the magnet electro-magnetic currents are set up in the vicinity of each pole. These electro-magnetic currents magnetize the particles of iron, and these in their turn take up a definite position in relation to the electro-magnetic currents. So in the far more complex phenomena of regeneration we may assume that certain definite currents of nutrition are set up from the axial gut out into the surrounding tissues. If these nutritive currents come in contact with embryonic tissue they cause the elements composing it to assume definite form and relations. Thus we have lost parts restored, and normal individuals produced from mere fragments. We do not wish to assert that currents setting out from the axial gut in various directions exert their specific action by purely mechanical means. On the contrary, the phenomena of regeneration are probably largely chemical in nature. Yet I see no reason for assuming that "head-forming stuffs" are conveyed into the region where a new head is being formed, or that pharynx-forming stuffs are conveyed into the region where a pharynx is produced.[1]

Of the internal conditions governing growth, that of the distribution of nutritive fluids is doubtless one of the most important. It will be found, I feel sure, that the direction and force of the nutritive currents play an essential part in determining the specific differentiation of tissues.

Before the development of blood-vessels in the growth of an embryo the nutritive currents must have a specific and important distribution, though just what this is remains to be determined for various embryos. That this non-vascular distribution of nutritive fluids may continue after the development of heart and blood-vessels is indicated by the very important work of Loeb (1893)[2] on Fundulus. On the other hand, the monstrosities known to occur when twins draw on a common placenta and one twin gets

[1] Sachs: Stoff und Form der Pflanzenorgane. Arbeiten der botanischen Instituts im Würzburg, 1882, ii, p. 457; also Loeb: *Loc. cit.* 1892.

[2] Loeb: Archiv für die gesammte Physiologie, 1893, liv, p. 525.

the major part of the blood, show how essential for the normal development of the embryo is a blood current of a given force and intensity.

IV. In spite of numerous attempts with various colored fluids I was unable actually to see in the living planarians the nutritive currents which I have assumed are set up in a determinate manner from the intestinal system. It is easy, however, to follow the "circulation" of food-stuffs within the intestines. The circulation of food in the intestines is brought about in part, at least, by the contraction of the body musculature.

As I have explained above, contraction of the whole animal causes the intestinal contents to be forced into that part of the intestine into which the pharynx enters, or posterior to which, when the pharynx is lacking, a new pharynx will be developed. I have called this the region of least intestinal pressure. By contractions of the whole worm defecation is thus produced in the normal worm. Contractions of this kind must not only force the intestinal contents, but also the nutritive fluids, towards the pharyngeal region.

The most common movements of the animal, however, are those which tend to impel it in a forward direction. Owing to the arrangement of the nervous system in relation to the musculature and to the periphery of the body, the movements of the animal as a whole are controlled by the most anterior parts of the nerve cords and the commissures uniting them. The movements set up from such a centre are either in the nature of waves of contraction which begin in the head and travel back towards the tail; or they consist of crawling movements — a slow extension of the body beginning at the head, the posterior part of the body remaining stationary — followed by a rapid contraction of the whole body towards the now attached head. This definite rhythmic slow expansion and rapid contraction must serve to set up definite nutritive currents in the animal. The smallest fragments capable of regeneration have an arrangement of nervous system and musculature which give rise to movements similar to those just described. It seems, therefore, fair to assume that nutritive currents are set up which have a definite relation to the intestinal system of the fragment, and which are among the essential conditions in the determination of the parts regenerated. Support is lent to this point of view by instances like that illustrated in Fig. 28. In this instance the movements of the parent worm seemed so to control the movements of the lateral slip that the latter was unable by definite

muscular contractions to set up nutritive currents of definite determinative value.

Rapidity of regeneration depends not on the position of the piece relative to the posterior or anterior extremities, but wholly upon the ease with which the conditions necessary for tissue regeneration can be set up. A tail piece may be developed into a perfectly formed worm many days before a small side piece from the region anterior to the pharynx has developed a head.

In the arrangement of the intestines and in the activity of the nervous system reside factors which serve to determine the arrangement of newly formed parts in the regenerating planarian. The genetic relations of the cells which go to form the new parts are difficult to follow. The new surface epithelium is derived from the old by cell migration over the cut surface followed by cell multiplication. The new intestinal branches are derived from the old, possibly here also by cell migration, as well as by cell multiplication. The cells which go to form the parenchyma, musculature, and nervous system, may in considerable part wander into their new positions from places occupied in the old tissue. The comparatively slight amount of karyokinesis that can be seen would indicate this. Methods which reveal great mitotic activity in the sexual cells of sexually mature worms reveal little of such activity in the cell mass which is differentiated into new parts. Morgan[1] has put much emphasis on morpholaxis, or the transposition of pre-existing tissues, taking place in the process of regeneration in the planarian. According to Keller (1894),[2] the cells which in fresh-water planarians during regeneration form the new parenchyma, musculature, and nervous system, arise from special undifferentiated cells, *Stammzellen*, embryonic in type. These may be distinguished from the ordinary parenchyma cells, and give rise either to the genital organs or to the tissues of regeneration. I have seen no morphological evidence of the existence of special cells of this nature in the Planaria maculata. The parenchyma cells, however, revert to a simple cell type, without branching processes, before becoming differentiated into new tissues.

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ON DIFFERENCES IN THE EFFECTS OF VARIOUS SALT-SOLUTIONS ON CILIARY AND ON MUSCULAR MOVEMENTS IN ARENICOLA LARVÆ. — I.

By RALPH LILLIE.

[*From the Hull Zoölogical Laboratory of the University of Chicago.*]

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I. Introduction.

CILIARY motion, muscular contractility, and karyokinesis have more than once within recent years been regarded merely as different forms of the same fundamental phenomenon of protoplasmic contractility. Further, it has been pointed out that all these motile activities seem to have in common a fibrillar structural basis, and the conception has finally been reached that wherever the property of contractility is specially developed, a specific fibrillar contractile tissue essentially identical with the archoplasm of dividing cells constitutes the active agency. Different forms of this contractile tissue are seen in the astral radiations and spindle fibres of dividing cells, the tails of spermatozoa, the fibrils of muscular tissue, and the cilia and intracellular fibrils of ciliated cells. In all these instances the motile activity depends on the special property of contractility possessed by the fibrils of this specific tissue, and the distinctive peculiarities of each form of activity depend merely on differences in the structure and arrangement of the contractile fibrils.[1]

A variety of criticisms may be urged against this conception. In the first place it is not certain that all contractile structures have a fibrillar basis. Colloids (solutions of egg-albumen, peptone, etc.)

[1] See *e.g.* Watasé: Origin of the Centrosome. Wood's Holl Biological Lectures, 1894.

fixed while under a condition of strain and subjected to the usual histological technique, are, as Hardy[1] especially has shown, extremely likely to exhibit a fibrillar structure remarkably similar to that shown by fixed and stained preparations of dividing cells and leucocytes; and we are thus led to consider seriously the possibility that the "contractile" astral fibres of dividing cells do not exist as such, but are similarly brought into being by the coagulating action of the fixative on the protoplasm while the latter is in the peculiar condition of strain that must necessarily accompany the process of cell-division. If then the basis of mitotic cell-division be not fibrillar, the fibrillar structure is not essential to protoplasmic movement, and the hypothesis of the necessary presence of a specific fibrillar substance common to all contractile structures is a baseless one.

Important criticisms have also been made from the more purely physiological standpoint. These are based chiefly on the differences observed in the effects of identical salt-solutions on different forms of contractility. If all forms of contractility are referable to the same structural basis, it is to be expected that all will be similarly affected by the action of the same chemical reagents, and that what favors one form of contractility will favor all others, and *vice versa*. This is far from being the case, however, as has been several times pointed out by Loeb in his recent studies of the effects of ions on different forms of protoplasmic activity.[2] Loeb found that sea-urchin blastulæ and gastrulæ were capable of swimming for forty-eight hours in solutions consisting solely of $MgCl_2$ and $CaCl_2$ in various proportions, and for a somewhat shorter time in solutions of NaCl and KCl. In such solutions, however, the muscular contractions of the swimming bell of Gonionemus were found to be completely impossible. Solutions of sodium and potassium chlorides, in which the proportion of potassium was so high as to completely inhibit the contractions of Gonionemus, readily allowed the development of Fundulus eggs up to the formation of the embryo. Here a long series of cell-divisions was possible in solutions that quickly destroyed all power of muscular contractility. Commenting on these facts, Loeb incidentally drew attention to the difficulty of reconciling them with the doctrine of the existence of a common structural basis for all forms of protoplasmic contractility.

The objection might perhaps be raised that although in the above

[1] Hardy, W. B.: Journal of physiology, 1899, xxiv, p. 158.

[2] Loeb, J.: This journal, 1900, iii, pp. 327, 383.

experiments proof is afforded that muscular contractility in Gonionemus has a different chemical basis from ciliary contractility in sea-urchin blastulæ, it is nevertheless not shown that in one and the same animal the two forms of contractility are essentially different. Such widely different organisms may well show differences in the properties of their respective contractile tissues, but in any one organism all contractile tissues may still quite possibly show the similarity of behavior required by the theory. Loeb has pointed out, however, that, in Fundulus embryos, solutions (consisting of NaCl and KCl in various proportions) which allow cleavage to proceed normally, interfere seriously with the heart-beat if the proportion of KCl exceeds a certain limit. In this instance we have a proof that in the same organism cell-division and muscular contractility are favored by entirely different combinations of ions, and that therefore their respective structural bases must differ widely from one another in chemical constitution. During the past summer at Wood's Holl I have been able to show in the experiments about to be described, that the same is true for ciliary and muscular movements in the larvæ of a polychæte Annelid, Arenicola cristata.

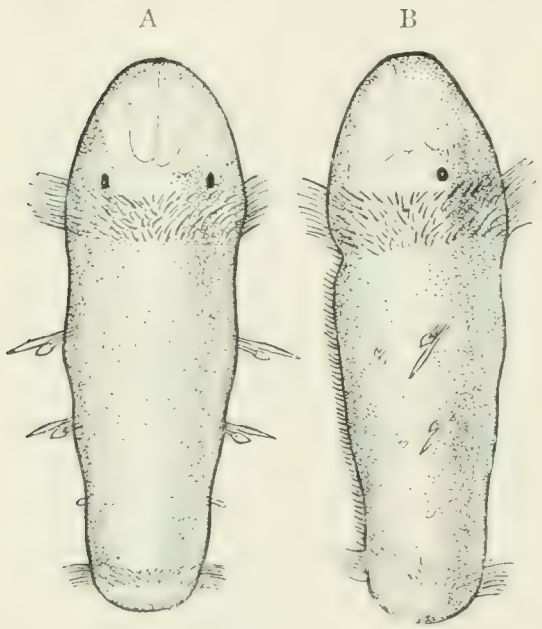


FIGURE 1. — Arenicola larvæ. A, from above. B, from one side. × 165.

Arenicola larvæ form remarkably favorable material for studies of this kind, both because of the ease with which they may be obtained in practically unlimited quantity, and because of their great vitality and the extreme constancy and definiteness of their reactions. They are easily obtained from the egg-strings if the latter be exposed to the light for two or three days in dishes of sea-water together with some Ulva for aeration. The free-swimming larvæ leave the strings at a swarming stage during which they show strong positive heliotropism accompanied by negative geotropism, as a result of which they collect at the surface of the water on the side of the dish nearest the source of light, and there become massed together in enormous numbers. The free-swimming positively heliotropic stage lasts for

two or three days and is succeeded by a burrowing and crawling stage in which the heliotropism becomes negative and the cilia are lost. The experiments about to be described relate to larvæ in the actively swarming stage.

A larva in this stage has a somewhat maggot-like appearance (Fig. 1), is cylindrical and somewhat elongated in shape and has already acquired three setigerous trunk-segments. The two usual ciliary rings of Annelid larvæ, prototroch and paratroch, are present, and in addition to these there is a median longitudinal band of cilia extending along the ventral surface. At the sides of the prostomium are two simple eyes, in the form of clumps of pigment granules on the surface of the brain. The internal organization is equally simple. Brain and ventral nerve cord are present in a simple condition; the intestine shows the usual three divisions but is still largely composed of yolk; the muscles are chiefly in the form of longitudinal fibrils applied to the inner surface of the body-wall; the setæ are moved in and out by special muscle-fibres attached to the inner ends of the seta-sacs.

We have thus in Arenicola larvæ organisms of small size and simple structure and of great constancy of reaction, yet already possessing well-differentiated contractile tissues essentially similar to those found in the adult. Their small size is of importance in that the exposure of a relatively large surface to the action of the solutions provides for a quick and thorough action on the contractile structures, so that the ions enter into immediate combination and act upon the structures in full strength and with relatively slight intermixture of foreign ions. The results are thus rendered peculiarly clear and definite, and any alterations in contractility immediately become evident. The precision of the results is further enhanced by the fact that many thousands of larvæ are acted upon at the same time in the same solution. The possibility of false conclusions being drawn from individual aberrancies of reaction is thus reduced to a minimum.

In the normal swimming movements of the larvæ both muscular and ciliary activities take part, and each of these can be shown to play a separate and distinct rôle. Propulsion is effected exclusively by the action of the cilia, which maintain a condition of continual and uniform activity in one direction; while the heliotropic orientation is a purely muscular phenomenon, dependent apparently upon the direct stimulating effect of light upon the muscles. This effect manifests itself in an increase of muscular tone under strong illumina-

tion; and as a natural consequence inequality of tone on the two sides of the body will result when one side is more strongly illuminated than the other. The muscles of the more strongly illuminated side being then in a state of greater contraction than those of the other, the result will be that the larva will become bent towards the source of illumination and will be carried towards the latter in a curved path by the action of its cilia. Equality of muscular tone on the two sides will result when both are equally illuminated, and this condition will normally be obtained only when the larva is so orientated that its long axis coincides with the direction of the light rays and its anterior end is directed towards the source of illumination. When so orientated, the action of the cilia will carry the larvæ towards the light. Any deviation to one side or the other will be immediately counteracted by an increase in the tone of the opposite side, resulting from the temporarily greater illumination to which it is exposed in consequence of the deviation. By this self-regulating mechanism a perfectly definite heliotropic orientation is retained. The resultant effect of the action of the two locomotor agencies is that the larvæ, when set free in the water, swim rapidly and at a perfectly uniform rate towards the source of illumination (Fig. 2) until stopped by the other side of the dish or some intervening obstacle. Slight lateral bendings and twistings, due apparently to momentary changes in muscular tone, cause each larva to describe a slightly sinuous path, but otherwise the heliotropic movement is perfectly direct and shows little or no individual variability. While swimming the larvæ rotate actively on their long axes.

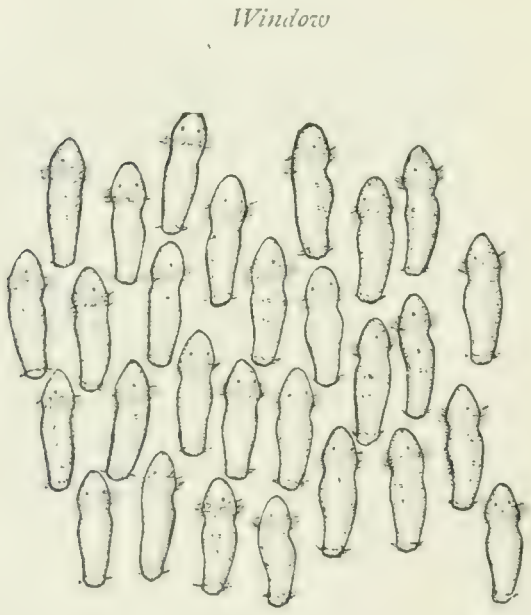


FIGURE 2. — Heliotropic swarming of larvæ, showing definite orientation with respect to direction of light rays. × 40.

During the swimming movements the setæ are retracted so as not to project beyond the surface of the body. They seem to come

into play whenever the larvæ are in contact with solid objects, and their action can be best observed in larvæ massed together at the light side of the dish. They are here continually being thrust forth and retracted, and in conjunction with the squirming movements of the body serve to push the larvæ along and disengage them from the contact of adjoining solid bodies or other larvæ. Later in life they play an important part in the formation of the tube and in the burrowing and crawling movements which then predominate.

I have given this short analysis of the normal locomotor movements of the larvæ in order to make clear the respective parts played by muscular and ciliary movement before proceeding to an account of the manner in which these activities (and hence the locomotor reactions of the larvæ) are affected by the salt-solutions. A fuller account will shortly be published of the normal reactions of the larvæ and the conditions that determine them.

II. Methods.

The salts employed were chlorides of the metallic elements most abundant in sea-water, sodium, potassium, calcium, and magnesium, in solutions approximately isotonic with sea-water, namely, $\frac{5}{8}n$ NaCl, $\frac{5}{8}n$ KCl, $\frac{10}{8}n$ $CaCl_2$, and $\frac{10}{8}n$ $MgCl_2$.[1] Each of these solutions was tried (1) singly, (2) in conjunction with each one of the others in mixtures of all possible proportions, and (3) in conjunction with *two* of the others in as many proportions as were practicable in the time available. The experiments were performed in finger-bowls which had previously been carefully washed in distilled water, and the examination under the microscope was made in shallow watch-glasses similarly treated. Usually each finger-bowl contained 100 c.c. of the solution whose action was to be tested. In transferring the larvæ from the sea-water to the solution, care was taken that as little sea-water as possible was transferred at the same time. There was generally no difficulty in this, since the larvæ in almost all cases were so closely massed

[1] The solutions were made by dissolving the weighed quantities of the chemically pure salts in distilled water. The salts, especially the magnesium chloride, were somewhat damp, so that the solutions were probably slightly below the chemically exact $\frac{5}{8}n$ or $\frac{10}{8}n$. However, since the aim in such experiments is simply to secure solutions *practically* isotonic with sea-water, such slight inaccuracies in the concentration of the solutions are of minor importance, and can in no way affect the qualitative results of the experiments.

together at the light side of the dish that portions of the mass, consisting of many thousands of larvæ, could be removed, with very little sea-water, and transferred to the finger-bowls. The larvæ could then be set free from the transferred mass with the aid of a pipette. Each finger-bowl thus contained thousands of larvæ. At intervals each bowl was examined and the condition of the larvæ carefully recorded. In addition, the immediate effect of the solutions on the larvæ was always directly observed in watch-glasses. In this instance the larvæ were freed of sea-water by taking advantage of their heliotropism, which within fifteen or twenty seconds after transference to the watch-glass caused them to collect in a mass at the light side. The sea-water could then be drained off and very completely removed by means of filter-paper. The solution to be tested was then added by a pipette in such a manner as to distribute the larvæ uniformly in the solution.

III. Effects of pure Sodium Chloride Solutions.

As had been anticipated from the results of Loeb's experiments (*loc. cit.*), pure $\frac{5}{8}\,n$ NaCl solutions were always found to be highly injurious; it was found, however, that within certain limits dilution of this solution delayed in a marked manner its poisonous action (compare Loeb, *loc. cit.*, and Miss Moore [1]). The following solutions were tried: —

TABLE I.

1. 100 c.c. $\frac{5}{8}\,n$ NaCl.
2. 80 c.c. $\frac{5}{8}\,n$ NaCl + 20 c.c. distilled water.
3. 50 c.c. $\frac{5}{8}\,n$ NaCl + 50 c.c. distilled water.
4. 30 c.c. $\frac{5}{8}\,n$ NaCl + 70 c.c. distilled water.

Addition of the first solution is instantly followed (1) by a sudden muscular contraction, which causes a shortening of the body to about half its normal length, after which there is a gradual relaxation; and (2) by an immediate and almost entire stoppage of ciliary motion so that swarming is cut short and the larvæ sink at once to the bottom of the vessel. Slow ciliary movements may persist for a short time, but at the end of one minute they have entirely ceased in all but a few exceptional larvæ which may show feeble movements for several minutes longer. In a few minutes a change in the appearance of the cilia is perceptible, due to a process of disorganization, apparently of the nature of liquefaction, which within a short time

[1] Moore, Anne: This journal, 1900, iv, p. 386.

(about ten minutes) usually results in the complete solution and disappearance of the cilia. Under a high power (4 mm. Zeiss objective) the process can be studied in detail. The first visible effect is a loss of sharpness of outline in the individual cilia; neighboring cilia lose their individuality and become fused, and if the cover-glass is moved at this stage the ciliary matter is seen to be drawn out into stringy filaments evidently of a glutinous consistency. As the liquefaction progresses the liquefied product takes on the form of minute round droplets which gradually flow away with the movements of the water. This alteration in physical consistency or state of matter is instructive in that it renders perfectly intelligible the destructive effect of NaCl solutions on ciliary motion. Apparently an excess of Na-ions in the ciliary tissues is followed by a loss of the physical consistency requisite for the movements, with the result that an immediate arrest necessarily occurs. The muscular movements on the contrary continue for some time after the addition of the solution. The initial contraction is followed by a gradual relaxation to the normal length accompanied by squirming motions and in-and-out movements of the setæ. In one experiment, after an interval of three and a half hours the majority of the larvæ were motionless and apparently dead; but a few individuals showed feeble movements then and for many hours afterwards, and in a small number feeble twitches were noticed after twenty-four hours in the salt-solution.

We see here a remarkable difference of effect in the action of pure NaCl solutions on ciliary and muscular forms of contractility respectively. Upon both the solution acts in a detrimental manner, but its action is much less injurious in the case of muscular than in the case of ciliary movement. The latter is almost instantly arrested, while muscular movement may continue for many hours before finally disappearing. Muscular movement will, in fact, continue for a much longer time in a pure NaCl solution than in a pure solution of any of the other salts, while exactly the reverse is true of ciliary movement, as will be shown later in detail. The injurious effect of the pure solutions is in both cases to be referred to the absence of the other ions necessary for contractility.

Both muscular and ciliary activity are conserved longer in the second and third of the above solutions than in the first. Solution 4 is more quickly fatal than any of the others, owing to the inability of the larvæ to withstand solutions of such low density, sea-water diluted $\frac{1}{3}$ being equally fatal. The following table shows the condition of

the larvæ in the different solutions after intervals of about one and two days:

TABLE II.

No.	Solution.	Condition of larvæ	
		After 26½ hours in solution.	After 48 hours in solution.
1	$\frac{5}{8}$ *n* NaCl.	Larvæ of granular and macerated appearance and almost all dead. A few exhibit slight muscular movements.	All dead and macerated.
2	80 c.c. $\frac{5}{8}$ *n* NaCl + 20 c.c. distilled water.	Larvæ of somewhat the same appearance as in 1, though maceration less marked; most larvæ show slow muscular movements. No ciliary movements.	Most are dead; maceration not so advanced as in 1. A few show slight muscular movements.
3	50 c.c. $\frac{5}{8}$ *n* NaCl + 50 c.c. distilled water.	Larvæ better preserved than in 2. Outline more regular, and granular appearance less marked than in 2. Slight ciliary movements still remain in perhaps a majority of larvæ, and nearly all show slow muscular movements.	Most are dead; a much larger proportion than in 2 show muscular movements, however. No ciliary movements.
4	30 c.c. $\frac{5}{8}$ *n* NaCl + 70 c.c. distilled water.	Dead. Maceration well advanced.	

We see from these results that the injurious effect of a pure-salt solution is less marked in dilute than in concentrated solutions. Sooner or later, however, in all pure NaCl solutions the poisonous effect appears, an effect which, since the natural medium also contains NaCl in amount approximately equal to that of a $\frac{5}{8}$ *n* solution, must be regarded as due to the absence in such solutions of certain other necessary salts.

IV. Solutions of two Chlorides.

The experiment was next tried of adding to the pure $\frac{5}{8}$ *n* NaCl solution small quantities of each of the other three salts, KCl, $MgCl_2$, and $CaCl_2$, with the aim of determining whether or not the presence of one of these in the solution would render the latter less injurious.

In these preliminary experiments 4 c.c. of each solution was added to 96 c.c. of the $\frac{5}{8}n$ NaCl. The solution 96 c.c. $\frac{5}{8}n$ NaCl + 4 c.c. $\frac{5}{8}n$ KCl was found to act in all visible respects similarly to the pure NaCl solution, and its injurious effects were equally marked. In the other solutions, however, a decidedly more favorable result was seen. In the solution 96 c.c. $\frac{5}{8}n$ NaCl + 4 c.c. $\frac{10}{8}n$ $MgCl_2$, although the immediate effect is a sudden contraction as in pure $\frac{5}{8}n$ NaCl, this passes off at once, and the larvæ straighten out and begin active swimming movements, which in some individuals even show a slight heliotropic tendency. The heliotropic movement (when present) ceases, however, in a few seconds, and the larvæ gradually settle to the bottom as a result of the gradual slackening of the ciliary movements. The ciliary movement, though considerably slackened, persists in all at least ten minutes, while in many it lasts for hours (in some for nineteen hours or even longer). It is clear therefore that ciliary movement is favored by the presence of this small amount of $MgCl_2$ in the solution. Addition of $CaCl_2$ is followed by even more striking results; when first added to the solution 96 c.c. $\frac{5}{8}n$ NaCl + 4 c.c. $\frac{10}{8}n$ $CaCl_2$ the reactions of the larvæ are, in fact, indistinguishable from those called forth in normal sea-water. The initial muscular contraction, so characteristic of the other solutions, does not appear, and the larvæ show the normal swarming heliotropic movements; they swim actively across the watch-glass and collect at the side next the window, and immediately swim back again when the watch-glass is turned about through 180°, and so on for perhaps two minutes or more. By this time the movements show evident signs of slackening, and at the end of five minutes the cilia are noticeably less active and heliotropism has largely disappeared. Both muscular and ciliary movements continue, however, in an enfeebled form for many hours longer. After nineteen hours a majority of larvæ show fairly active muscular contractions, and a considerable number still retain ciliary activity, in some to a degree sufficient to admit of slow swimming movements. The presence of the small amount of calcium has therefore delayed for many hours the ciliary disorganization caused by pure NaCl solutions, and has enabled the larvæ to retain for a greatly increased period the power of muscular contractility.

The next step was to ascertain the effect of varying the proportions of the two salts, in the hope that a more definite light might thereby be thrown on the respective parts played by each ion in the activities

under consideration. Graded series of solutions of two salts were used in such experiments. In such series the quantity of one salt gradually decreases and that of the other increases from one end of a series to the other; the extremes are therefore represented by pure or almost pure solutions of one salt. The specific action of each constituent can thus be determined very accurately by comparing the effects of solutions in which it is present to a greater or less degree. There is seen on passing along the series a gradation of effect in close correspondence with the gradual change in the relative proportions of the two salts.

The first series of experiments was on mixtures of solutions of NaCl and $CaCl_2$. The following table shows the solutions employed, together with the observed condition of the larvæ after intervals of approximately one, two, and three days in the solutions.

In studying the immediate action of these solutions, an interesting gradation of effect is seen in passing in order from solution to solution. The effect of even the first member of the series (containing a mere trace of Ca) is strikingly different from that of a pure NaCl solution. The addition of Solution 1 is immediately followed by heliotropic swarming movements, which, however, in solutions with so low a Ca-content, very soon cease (in most larvæ within 30 seconds or less); and at the end of five minutes, while a few exceptional larvæ may still show slow swimming movements, in the great majority ciliary movement has become greatly slowed and in many entirely arrested. If larvæ are treated with a solution of a slightly increased Ca-content, viz. No. 2 of the above series, a very decided improvement is observed. An active and rapid heliotropic swarming immediately sets in and continues in many larvæ for three minutes or even longer, while irregular swimming movements without orientation continue for a considerable space of time afterwards. After four hours ciliary movements have mostly ceased in this solution, but still feebly persist in a few cases, while in Solution 1 after the same interval no trace of movement remains. With a still further increase in the proportion of $CaCl_2$ the favorable effect on ciliary motion is increased, until finally an optimum proportion is reached, after which a further increase becomes relatively unfavorable. The optimum effect seems to be gained when the proportions are approximately those of Solution 9 in the above table (80 NaCl + 20 $CaCl_2$). In this solution the ciliary activity and the heliotropic swarming remain

TABLE III.

No.	Proportion of salts in solution.	Condition of larvæ		
		After about 20 to 21 hours in solution.	After about 2 days in solution.	After 66–68 hours in solution.
1	NaCl 99 $CaCl_2$ 1	Most are dead, shrunken, macerated. A few slight muscular movements. No ciliary movement.	Most are dead and shrunken. No cilia. A few slight muscular movements. (50 hours.)	Almost all dead and macerated. A few feeble contractions.
2	NaCl 98 $CaCl_2$ 2	Similar to 1. A few show slight ciliary movements.	Most are dead and shrunken. No cilia. Slight muscular movements in a considerable number.	Like 1; no movements seen, however.
3	NaCl 97 $CaCl_2$ 3	Similar to 2.	Shrinkage marked. A very few show feeble ciliary movements. Muscular movements, more active than in 2, are shown by most.	Like 1. A few show feeble muscular movements. No cilia.
4	NaCl 96 $CaCl_2$ 4	Similar to 3.	Similar to 3. No ciliary movements seen.	A somewhat larger proportion than in 3 show muscular movements.
5	NaCl 95 $CaCl_2$ 5	More favorable than 4; smaller proportion dead; larger proportion show ciliary activity, which in one or two suffices for slow swimming movements.	Fair number show ciliary activity. Many show muscular activity without ciliary movement.	Similar to 4. No ciliary movement.
6	NaCl 92.5 $CaCl_2$ 7.5	Similar to 5.	Fair number show ciliary activity, though majority do not. Muscular activity more widely distributed than ciliary activity.	Similar to 5, but larvæ somewhat better preserved; maceration less marked.
7	NaCl 90 $CaCl_2$ 10	Most are living, though many are badly shrunken and macerated. Cilia mostly active, in some sufficiently for slow swimming movements.	After 44 hours: ciliary activity retained in many larvæ; some show slow swimming movements. Muscular movements in many larvæ whose cilia have stopped.	A few larvæ show feeble contractions at intervals. No ciliary movement.

TABLE III — *continued.*

No.	Proportion of salts in solution.	Condition of larvæ		
		After about 20 to 21 hours in solution.	After about 2 days in solution.	After 66–68 hours in solution.
8	NaCl 85 $CaCl_2$ 15	Essentially same as 7. Cilia perhaps more active.	Similar to 7.	All are dead, shrunken, macerated.
9	NaCl 80 $CaCl_2$ 20	Similar to 8.	Larvæ generally alive; a few are only slightly altered and swim about. Most are shrunken.	Similar to 8.
10	NaCl 70 $CaCl_2$ 30	Most are living. Many show muscular movements. Cilia similar to 9; a few slowly swimming.	After 45 hours: maceration well advanced.	
11	NaCl 60 $CaCl_2$ 40	Maceration further advanced than 10. All show ciliary activity. Muscular contractions less pronounced than in 10.	Larvæ completely disintegrated.	
12	NaCl 50 $CaCl_2$ 50	Maceration marked. Most larvæ show ciliary activity; some are swimming. Muscular movements almost entirely absent.	All are dead and macerated.	
13	NaCl 40 $CaCl_2$ 60	Macerated; in many cases larvæ have become mere heaps of granular detritus; in *all*, however, ciliary activity persists, in some to a considerable degree.	All are dead and macerated.	
14	NaCl 30 $CaCl_2$ 70	Maceration further advanced than in 13; almost all are reduced to shapeless granular lumps; but in most the cilia still move feebly.	All are dead and macerated.	

TABLE III — *concluded*

No.	Proportion of salts in solution.	Condition of larvæ		
		After about 20 to 21 hours in solution.	After about 2 days in solution.	After 66–68 hours in solution.
15	NaCl 20 $CaCl_2$ 80	Condition similar to 14, but maceration still further advanced; the lumps corresponding to larvæ are of looser consistency. In a large proportion feeble ciliary movements persist.	All are dead and macerated.	
16	NaCl 10 $CaCl_2$ 90	Larvæ all reduced to masses of loose granular detritus. In a considerable number slight ciliary movements persist.	All are dead and macerated.	
17	$CaCl_2$ 100	Disintegration as in 16; no signs of life or ciliary movements.	All are dead and macerated.	

almost normal at the end of five minutes; and although the cilia slacken in course of time, they may retain their activity in some larvæ sufficiently to permit of slow swimming movements for as long as forty-four hours.

The power of heliotropic response, however, even with the most favorable proportions of NaCl and $CaCl_2$, disappears very much earlier; the swimming movements, at first definitely orientated, soon become irregular and uncoördinated, and after at most ten minutes have in all larvæ completely lost their heliotropic character. They may persist in an irregular form for hours after this, but the sensitivity of the muscles to a light-stimulus seems to have disappeared, and the swimming movements thenceforth exhibit no orientation. That this effect is due to an alteration in the condition of the muscles themselves, and not to changes in the nervous system or in the general sensibility, is indicated by the fact that a similar lack of coördinated movement quickly makes its appearance whenever solutions are used that are injurious to muscular activity (whether through an insufficiency of Na-salts or the presence of K- or Mg-salts), and this

the more rapidly the more injurious the solution used. These facts and others that will be presented soon, afford a strong confirmation of the view expressed earlier in this paper that heliotropic orientation is a result of the direct stimulation of the muscles by the light rays.

Solutions 7 and 8 (with 10 c.c. and 15 c.c. of $CaCl_2$ respectively) preserve ciliary movement and the power of heliotropic response almost as well as Solution 9. In the first-named solution (No. 7) muscular activity after an interval of 44 hours is found to be somewhat better preserved than in the other two. This indicates that the optimum solutions for muscular movement do not coincide in their proportions with the optimum for ciliary movement, but contain less Ca; and this was indeed found to be the fact. In Solution 6 (92.5 c.c. NaCl + 7.5 $CaCl_2$) it was found that after 50 hours the very considerable number of larvæ still living showed decidedly more muscular than ciliary activity; the latter indeed persisted feebly in only a small number. In Solution 4 after the same interval of time a considerable proportion also showed slow muscular movements, but no ciliary movements were observable. In Solution 3 muscular movements were more active than in 4, while in Solution 2, though muscular movements were made by a very considerable number of larvæ, there was no trace of ciliary movements. After a further interval of eighteen hours (68 hours after addition of solution) a few larvæ were found in Solutions 1, 3, 4, 5, and 6, which still showed feeble muscular contractions, although the great majority were dead and partially macerated. A very few in Solution 7 showed at long intervals slight contractions after a period of 66 hours, but in Solutions 8 and 9 (the most favorable for cilia) no traces of movement were seen, and the larvæ were all dead and of a shrunken and macerated appearance.

It is clear from these facts that the solutions most favorable to ciliary movement are less favorable to muscular movement than solutions with a decidedly lower Ca-content; in other words, each form of contractility has its own characteristic optimum solution. The most important general result, however, is that for both forms of activity solutions containing both Na and Ca in certain proportions are much more favorable than pure NaCl solutions; the effect is more striking in the case of ciliary movements, which are almost completely impossible in the pure Na-solutions, while muscular movements may continue in such solutions for some time. We shall see later that muscular movements are impossible in pure

$\frac{10}{8}$ *n* $CaCl_2$ solutions, which nevertheless will sustain ciliary movement for a very considerable period.

After the optimum proportion is reached further increase in the proportion of Ca is followed by a corresponding decrease in the favorable action of the solutions, a decrease which becomes more and more marked as the end of the series is approached, until finally in the pure $CaCl_2$ solution the injurious effect of a pure solution of a single salt again becomes manifest, though in a manner almost exactly opposite to that shown by a pure NaCl solution. The immediate effects called forth by Solutions 10, 11, 12, and 13 are nearly alike; in all there is at first observed the typical heliotropic swarming which, however, lasts for a noticeably shorter time than in Solutions 7, 8, and 9, before referred to, and generally for a shorter time in each solution than in the one immediately preceding it. This shortening in the time during which heliotropism is shown becomes more and more marked as the proportion of Ca is increased. It is a direct result of the increasingly unfavorable effect on muscular movement which follows a decrease of the Na and an increase of the Ca in the solution. That this is the true explanation is shown by a comparison of the condition of larvæ kept an equal time in Solutions 10, 11, 12, and 13. After about four hours, muscular movements are found to have disappeared in the larvæ of Solution 13 while still present in the others. After 21 hours the larvæ in Solution 10 show slight muscular movements in a fair proportion of cases, in Solution 11 the movements are decidedly less marked, in Solution 12 they are almost completely absent and in Solution 13 they are entirely absent. In all these solutions, however, ciliary activity is well retained. In Solution 14 muscular rigidity appears still more quickly and becomes evident in a few minutes; in the succeeding solutions this effect is even more marked, and in the pure Ca-solutions rigidity begins its appearance almost immediately and is usually complete within two or three minutes. In direct correspondence with these facts we find that the heliotropic response becomes less and less marked and lasts for a shorter and shorter time as we descend the series, until finally in the pure Ca-solution its appearance is only momentary, lasting for not more than two or three seconds.

The persistence of active ciliary movements in solutions with a large Ca-content gives rise, so soon as muscular rigidity has begun to appear, to a curious and very characteristic change in the distri-

bution of the larvæ. Instead of sinking uniformly to the bottom as their power of orientation disappears, they become collected in numerous small clumps or bunches scattered irregularly over the bottom of the watch-glass (Fig. 3). Each clump consists of a large number of larvæ. If the larvæ in such a watch-glass be again stirred up and uniformly distributed by means of a pipette, they are found to swim actively so long as they remain suspended in the water; but they soon sink at random to the bottom and again become clumped together in the manner described. The phenomenon first becomes noticeable in Solution 14, where it comes on relatively slowly and begins its appearance only after the larvæ have been three or four minutes in the solution. The muscular movements in this solution almost completely disappear in about five minutes, and the clumping then becomes strikingly evident. In the succeeding solutions, however, the clumping appears more rapidly; in Solution 16, and especially in Solution 17, the majority of the larvæ take on this peculiar distribution within less than a minute.

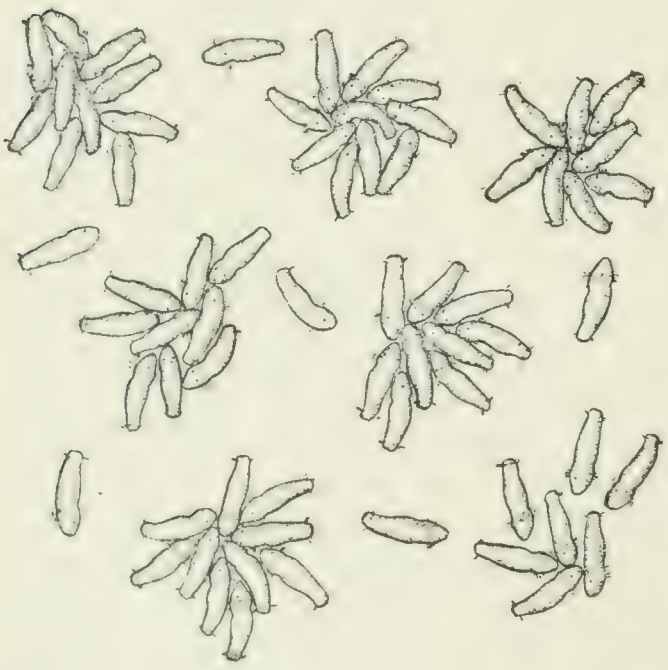


FIGURE 3 — Clumping and loss of orientation induced by solutions of $CaCl_2$, $MgCl_2$, etc. × 25.

Upon closer examination the conditions upon which this curious phenomenon depends become at once evident. As intimated above, the effect is found to depend on the marked difference in the action of such solutions on muscular and ciliary movements respectively. The former are rapidly and completely checked, while the latter continue with almost undiminished activity for a considerable period after muscular movement has entirely ceased. Under the microscope the on-coming of the phenomenon can be seen best in such a solution as No. 16. Immediately upon the addition of the solution a sudden con-

traction and shortening of the body takes place, followed by a relaxation and, in many larvæ, by heliotropic swarming. The muscular movements, which during the first few seconds appear almost normal, are seen gradually to become less active; the squirming movements soon present a stiff and labored appearance, and the setæ gradually cease their in-and-out movements. Coincidentally with these changes all orientation, heliotropic and otherwise, disappears and the swarming becomes undirected. A few seconds later rigidity has become almost complete, and the larvæ are carried about passively and at haphazard by the ciliary movements, which preserve vigorous and almost undiminished activity. The larvæ while in this state swim about like rigid ciliated cylinders. Many of them are fixed in a permanently bent condition and follow continuous circular paths in the direction of the body-curvature. Being specifically slightly heavier than the solution, they gradually settle to the bottom. Arrived here, each larva is carried in a chance direction by its ciliary action until it meets with an obstacle, generally another larva, to which it slightly adheres, from its inability to use its setæ or to make the other muscular movements necessary for detachment. Little groups of larvæ are thus quickly formed. These are continually increased by the accession of the larvæ still capable of motion, and eventually all become collected in the clumps or groups formed in this purely casual way. If after the larvæ are thus aggregated the solution is stirred with a pipette so as to again bring about a uniform distribution, the random swimming and clumping reappear as before. The experiment may be repeated so long as the cilia retain a sufficient degree of activity for swimming movements.

The time during which ciliary action persists in solutions with a high Ca-content (from Solution 10 on) becomes in general less and less as the proportion of Ca increases and is least in the pure $CaCl_2$ solution. This may be seen on comparing the condition of the larvæ in Solutions 13 to 17 after an interval of 21 hours, as recorded in the above table, which shows clearly that the solutions become progressively more unfavorable as the proportion of Ca increases. Here we see also the remarkable power which Ca has of retaining cilia in an active condition even after the remaining tissues have undergone an almost complete disintegration. Nothing, I think, can demonstrate more forcibly than such facts the essentially specific nature of ciliary contractility. Even after ten minutes or more in a pure $CaCl_2$

solution most of the larvæ, when shaken up, are capable of quite active swimming movements; and feeble vibrations of the cilia continue of course for a long time afterwards. Similar phenomena are seen, as will shortly be described, in pure solutions of $MgCl_2$ and in other solutions where muscular contractions are equally impossible.

Solutions containing $MgCl_2$ and NaCl in graded proportions were next tested. The following table gives the composition of the solutions used, together with the condition of the larvæ at the stated intervals.

In general these solutions act very similarly to those of the Na and Ca series. Mg seems, however, to have an even more decidedly favorable effect than Ca on ciliary action, while on muscular movement its effect is noticeably more injurious. Muscular activity never becomes perfectly normal in these solutions, and therefore heliotropic swarming does not appear; and the rigidity consequent on loss of contractility appears decidedly earlier than in the Ca series. However, the presence of a small amount of Mg in a NaCl solution enables muscular contractility to continue for a much longer period than would be possible in a pure NaCl solution, and in this respect the action of the two ions seems similar. Apparently Mg can to a certain extent replace Ca in the tissues, although the presence of the latter, if only in small amounts, is indispensable for normal activity. We shall see later that solutions consisting chiefly of Mg and Na salts but with a relatively small amount of Ca (*e.g.*, NaCl, 40 c.c. + $MgCl_2$, 55 c.c. + $CaCl_2$, 5 c.c.) are capable of preserving larvæ in a living condition for a much longer period than solutions of Mg and Na without Ca. In general, however, the respective parts played by the two elements seem closely similar, as might perhaps have been anticipated from their close chemical relationship.

The table shows many points of comparison with the Na and Ca series. Solution 2, apparently the most favorable solution for muscular movement, contains, it will be noticed, a much smaller proportion of Mg than does the solution most favorable for ciliary movement, which contains from 10 to 20 parts per 100 of the $MgCl_2$ solution. The characteristic differences in the action of the two metals soon become evident. Ciliary movements are retained decidedly better in these solutions than in the corresponding Ca solutions, while muscular movements are unfavorably affected and immediately lose

TABLE IV.

No.	Proportion of salts in solution.	Condition of larvæ			
		After 16 hours in solution.	After 28–29 hours in solution.	After 42 hours in solution.	After 65 hours in solution.
1	NaCl 99 $MgCl_2$ 1	Most are dead; a few show slight muscular movements. Ciliary action almost entirely absent.	Many show muscular movements; a *few* show slight ciliary movements. Shape of body generally unaltered.	Most are dead and slightly shrunken. No ciliary action. A few show feeble muscular movements.	All are dead. Shape well preserved.
2	NaCl 97.5 $MgCl_2$ 2.5	Similar to 1; more show muscular movements.	Like 1, but much larger proportion show ciliary activity, sometimes very marked.	Larger proportion living than in 1. A few show slight ciliary movements.	Most are dead; a few show slight ciliary movements.
3	NaCl 95 $MgCl_2$ 5	Slight ciliary motions in a few. Muscular movements in a few more.	Majority show ciliary and muscular activity. Shape almost normal.	Fair number show ciliary movements, in some sufficient for slow swimming. Fair number show muscular movements, though fewer than 2.	Same as 2.
4	NaCl 90 $MgCl_2$ 10	Ciliary activity in majority, in some sufficient for slight swimming movements; a number show muscular movements.	Decidedly more favorable than 3. Ciliary activity sufficient in many cases for very active swimming movements. Muscular activity also well preserved.	Ciliary activity decidedly more pronounced than in 3, and shown by most larvæ. Considerable number show very lively swimming movements. Larvæ *rigid*; no muscular movement.	Fair proportion show ciliary movements, which in a few suffice for slight swimming movements.
5	NaCl 80 $MgCl_2$ 20	Similar to 4. Ciliary movements perhaps more pronounced. Shape normal.	Generally similar to 4, but muscular activity not so well retained; larvæ more rigid and muscular movements slow and stiff. Shrinkage apparent.	Smaller proportion than in 4 show ciliary movement. Maceration and shrinkage evident. No muscular movement.	Smaller proportion than in 4 show ciliary movement. Maceration well advanced.

TABLE IV — (*continued*).

No.	Proportion of salts in solution.	Condition of larvæ			
		After 16 hours in solution.	After 28–29 hours in solution.	After 42 hours in solution.	After 65 hours in solution.
6	NaCl 70 $MgCl_2$ 30	Cilia more active than in 5; fair number show swimming movements. Rigidity noticeable; muscular movements slight. Body somewhat shrunken from cuticle.	Larvæ shrunken and maceration beginning. Rigidity marked; a few stiff muscular movements. Ciliary movements not so well preserved as in 5.	Disintegration further advanced than in 5. Most are dead; a very few show feeble ciliary movements. No muscular movements.	Maceration furtheradvanced than in 5. No movements visible.
7	NaCl 60 $MgCl_2$ 40	Similar to 6. Swimming movements somewhat more active than in 6. Shrinkage and commencing maceration plainer than in 6.	Larvæ shrunken and considerably macerated, and cuticle distended. Most are dead; a few show ciliary activity. No muscular movements.	All are badly disintegrated.	
8	NaCl 50 $MgCl_2$ 50	Shrinkage and commencing maceration marked. Ciliary activity as in 7. No muscular movement.	Apparently all dead, macerated and shrunken.		
9	NaCl 40 $MgCl_2$ 60	Cilia active. Maceration and shrinkage further advanced. No muscular movement.	As in 8. Shrinkage and maceration further advanced. One larva seen with still very faint ciliary movement.	All are dead and disintegrated.	
10	NaCl 30 $MgCl_2$ 70	Body much shrunken and macerated, and cuticle distended. Ciliary activity less than in 9. No muscular movement.	All are dead and disintegrated.		

TABLE IV — (*concluded*).

No.	Proportion of salts in solution.	Condition of larvæ			
		After 16 hours in solution.	After 28–29 hours in solution.	After 42 hours in solution.	After 65 hours in solution.
11	$NaCl$ 20 $MgCl_2$ 80	Body like 10, but disorganization more marked. Ciliary movements almost entirely absent. No muscular movement.	All are dead and disintegrated.		
12	$NaCl$ 10 $MgCl_2$ 90	Similar to 11. A few very feeble ciliary movements.	All are dead and disintegrated.		
13	$MgCl_2$ 100	Badly macerated. No ciliary or muscular movement. Apparently quite dead and disintegrated.	Disintegration more complete than in the above. Larvæ reduced to granular detritus within the swollen cuticle.		

coördination, even though the power of contractility may persist in an irregular form for a very considerable period.

When the immediate action of the solutions is studied in the watch-glasses a gradation of effect is seen on passing in order from solution to solution, similar to that observed in the case of the Ca series. Solution 1 acts similarly to a pure NaCl solution (although decidedly less injuriously) in quickly checking ciliary activity, which largely ceases within about three minutes after the solution is added. In Solution 2 cilia remain active for a much longer time, and in Solution 3 and the following solutions the ciliary activity may persist for hours, and the larvæ may swim about irregularly, often for a considerable period, the length of which varies according to the nature of the solution. The most favorable proportions are those of Solution 4 (as seen from the table), and the period of activity diminishes on passing along the series in either direction from Solution 4. Muscular movement, however, as above stated, never assumes a normal character in any of these solutions. The immediate action of the earlier

members of the series, containing a moderate amount of Mg (Solutions 2–6), is to cause a strong contraction and shortening of the body followed by an increased activity, expressed in vigorous bendings and squirmings which persist for several minutes and cause the swimming larvæ to take very tortuous and irregular paths. In Solutions 4, 5, and 6, the early swimming movements are typically of a wheeling or circular kind, due apparently to a persistence of the larvæ in the bent condition for several seconds before the reversed contraction takes place. In the following solutions also there is immediately observed a vigorous initial contraction which results in the above mentioned shortening of the body, after which relaxation and the typical squirming movements appear. Relaxation occurs in a second or two in the earlier solutions, but in the later solutions it appears more slowly and when Mg greatly preponderates (as in Solutions 12 and 13) relaxation may be almost immediately followed by muscular rigidity. In accordance with this it is found that as the proportion of Mg increases the time during which the active bendings persist becomes less and less. In Solution 8 larvæ become noticeably stiffer in their movements within $2\frac{1}{2}$ minutes after the addition of the solution, and within 5 minutes rigidity is complete. In Solution 9 rigidity comes on more quickly, in Solution 10 still more quickly, in Solutions 11, 12, and 13 within a few seconds, and in the pure $MgCl_2$ solution it appears most quickly of all.

Rigidity frequently appears when the larvæ are in a bent condition, as in the Ca solutions, but still more frequently in the Mg solutions, apparently in consequence of the more marked lateral bendings called forth, and the more rapid action of Mg in arresting the muscular movements. In the Mg solutions the bent and rigid larvæ, carried about by the persistently active cilia, soon become collected in clumps at the bottom of the watch-glass, exactly as in the Ca solutions (Fig. 3). Clumping, in fact, appears in any solution that deprives the muscles of contractility without immediately interfering with ciliary activity; and the effect is particularly well shown in the later members of this series of solutions.

I pass now to the effects of solutions of sodium and potassium chlorides. In this series of experiments it soon becomes evident that K also differs greatly in its effects on muscular and on ciliary activities. Towards the former potassium appears markedly antagonistic, its presence even in small quantity being sufficient to quickly destroy all power of movement; while towards ciliary movement its action is

much less unfavorable, since even in a pure KCl solution active ciliary motion may continue for a very considerable time. The following table shows the series of solutions employed and the condition of the larvæ after an interval of four hours.

TABLE V.

No.	Solution.	Condition of larvæ after 4 hours in solution.
1	99 c.c. NaCl + 1 c.c. KCl	Dead. No trace of cilia.
2	97.5 c.c. NaCl + 2.5 c.c. KCl	Similar to 1.
3	95 c.c. NaCl + 5 c.c. KCl	Similar to 2.
4	90 c.c. NaCl + 10 c.c. KCl	Similar to 3. A few traces of the ventral ciliary band remain.[1]
5	80 c.c. NaCl + 20 c.c. KCl	Like 4. Traces of ventral band more pronounced. No traces of prototroch or paratroch.
6	70 c.c. NaCl + 30 c.c. KCl	Ventral cilia largely intact. Traces of prototroch and paratroch in a few larvæ.
7	60 c.c. NaCl + 40 c.c. KCl	Like 6. Cilia better preserved.
8	50 c.c. NaCl + 50 c.c. KCl	Like 7. Cilia better preserved, but still largely dissolved.
9	40 c.c. NaCl + 60 c.c. KCl	Cilia as in 8; better preserved.
10	30 c.c. NaCl + 70 c.c. KCl	All cilia present in most larvæ.
11	20 c.c. NaCl + 80 c.c. KCl	Cilia present and well preserved.
12	10 c.c. NaCl + 90 c.c. KCl	Cilia present and well preserved.
13	100 c.c. KCl	Cilia present and well preserved.

None of the cilia, however, retained any traces of activity.

All of these solutions are, relatively to those of the last two series, decidedly injurious, and the larvæ survive their action for not more than 3 or 4 hours at most. The immediate effect of the earlier members of the series is very similar to that of a pure NaCl solution. In the case of Solution 1 there occurs at once a violent contraction and shortening of the body, followed by a slow relaxation and the appearance of feeble muscular movements which persist for some time. Ciliary action is almost immediately suppressed; and in a few minutes the cilia liquefy and dissolve, precisely as in a pure

[1] The ventral band is composed of shorter and thicker cilia than the prototroch and paratroch, and in correspondence with this its activity in unfavorable solutions always persists longer than that of the two ciliary rings.

NaCl solution. These effects are seen especially in Solutions 1, 2, and 3; in Solution 4 the muscular movements are more quickly arrested than in the three preceding solutions, but in other respects the effect is similar. As the proportion of K increases on passing down the series, the destruction of the cilia becomes less and less evident, as may be seen by referring to the table, which records the condition of the larvæ about four hours after the solutions were added. In correspondence with this it is found that the period during which the cilia remain capable of activity becomes longer as the proportion of K increases. In Solution 9 a few larvæ are still capable of slow swimming movements after 5 minutes in the solution. In Solution 10 this is true to a still greater degree. Here a slight tendency to bunching first becomes evident, a tendency which becomes successively more marked in Solutions 11, 12, and 13. In the pure KCl solution (No. 13) the cilia, strange to say, are less interfered with than in any of the other solutions of the series. The larvæ contract violently (as in all solutions of the series) on the first contact with the solution, but the cilia preserve for the first few minutes almost unchecked activity, and the larvæ thus carried about quickly become bunched together in the typical manner. Seven minutes afterwards the cilia are still vigorously active, though somewhat less so than at first; the larvæ still rapidly bunch, however, when stirred in the solution. The ciliary activity gradually becomes feebler, and after an hour's interval has largely ceased, although in many larvæ the ventral band may remain feebly active.

Muscular movements are quickly checked in all the solutions from No. 4 on, although slight twitches may persist for a few minutes, especially in the solutions containing little K. In Solutions 11, 12, and 13 rigidity appears very rapidly, and in Solution 13 it is complete within a few seconds after the initial contraction. The fact that even a very small amount of K quickly destroys all power of muscular movement, seems to indicate that this ion has a specifically injurious action, and that the effect observed is not due (as in the case of the Ca solutions) to an insufficiency of Na-ions; for it appears even in Solution 4 which contains a high proportion of Na. This specifically injurious effect has been also observed in the case of vertebrate muscles by Greene,[1] Loeb,[2] Miss Moore[3] and others,

[1] Greene: This journal, 1898, ii, p. 82.

[2] Loeb, J.: Festschrift für Fick. Braunschweig, 1899, p. 101.

[3] Moore, Anne: *Loc. cit.*

and by Loeb also for Gonionemus.[1] In the case of Arenicola larvæ I have found it evident in all solutions that contain more than a very small proportion of the salt, whatever their other constituents may be.

V. Solutions containing no Sodium.

The removal of Na from the medium is followed by the almost complete loss of the activities which require its presence in large amount, such as the power of muscular movement. Sodium, while more necessary to some forms of vital activity than to others, plays such an essential part in all that the entire absence of its salts is generally speaking more quickly detrimental than the absence of any of the other salts normally present. Yet certain activities, especially ciliary movement, can be preserved for very considerable periods in the absence of Na by the use of favorable proportions of other salts. The three following series of experiments, especially those with mixtures of $MgCl_2$ and $CaCl_2$, will illustrate this.

Solutions with Ca and K, but containing no Na, were first tested. Needless to say, muscular contractions were quickly arrested in all these solutions. Ciliary activity, however, was everywhere possible, although at the end of an hour it had mostly ceased in all but a few solutions at the Ca end of the series. Bunching therefore was very characteristic and appeared quickly. In accordance with the more favorable action on cilia of Ca as compared with K, it was found that ciliary movement lasts longest in the solutions near the Ca end of the series. The *pure* Ca solution, however, it is important to notice, acts decidedly less favorably than the solution immediately preceding it (with 97.5 c.c. $\frac{10}{8}n$ CaCl + 2.5 c.c. $\frac{5}{8}n$ KCl). In this latter solution, which is the most favorable of all, a good many larvæ were found after an interval of three quarters of an hour still capable of slow swimming movements. Here again the favorable influence of the presence of another salt in diminishing the injurious action of the pure solution is apparent.

One characteristic difference of effect between solutions at opposite ends of the series may be especially mentioned as demonstrating in a convincing manner the specifically injurious action of K on muscular contractility. The solutions at the K end cause, after the initial muscular contraction, an almost instant stiffening of the larvæ. A few feeble twitches may persist for two or three minutes, but apart

[1] Loeb, J.: This journal, 1900, iii, pp. 327, 383.

from this, rigidity is almost complete within a few seconds after contact with the solution. In solutions at the Ca end, on the other hand (with 10 c.c. $\frac{5}{8}$ *n* KCl + 90 c.c. $CaCl_2$, 5 c.c. KCl + 95 c.c. $CaCl_2$, 2.5 c.c. KCl + 97.5 c.c. $CaCl_2$, and pure $\frac{1.0}{8}$ *n* $CaCl_2$, respectively), the loss of contractility is more gradual. In the second of the solutions mentioned, muscular squirmings and movements of the setæ continue in an almost normal manner for perhaps 30 seconds and then gradually cease. In the third solution the movements are at first almost normal, becoming noticeably stiffer only after an interval of about one minute, and rigidity is not complete until two minutes or more have elapsed. In the pure $CaCl_2$ the muscular movements approach still closer to the normal, and many larvæ show at first a decided heliotropic tendency. Rigidity, however, gradually appears and is complete in three or four minutes. Since Na, the ion most necessary to muscular contractility, is equally absent in all these solutions, the above difference of effect can be referred only to a specifically injurious influence exercised by the K-ions on the contractile elements.

Solutions of $MgCl_2$ and KCl were found to give essentially the same results, with the characteristic differences that ciliary movement lasts longer than in the solutions just referred to, and that the muscular movements at the Mg end of the series, while lasting decidedly longer than at the K end, approach much less closely to the normal than in the corresponding solutions of the Ca and K series.

Mixtures of $MgCl_2$ and $CaCl_2$ favor ciliary activity, as was to be expected, while they quickly check muscular movements (though less rapidly than the above K solutions). The following table gives the record of a series of experiments with mixtures of these salts.

The immediate effects of these solutions may be briefly described. The solutions all favor ciliary activity, while they quickly interfere with muscular movement, so that the bunching or clumping phenomena are particularly striking. An interesting gradation of effect is observable in passing from one end of the series to the other. When the larvæ are placed in Solution 1 they exhibit for the first few seconds almost normal activity, and swim towards the light in the usual way. Heliotropism soon disappears, however, as muscular contractility becomes affected; and rigidity is almost complete within about two minutes. Ciliary activity, on the other hand, is apparently unaffected at first and remains almost as intense as ever, with the

TABLE VI.

Larvæ placed in the solutions at 11.50 A.M., July 7.

No.	Solution.	Condition of larvæ.
1	95 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 5 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	2.41 P.M.: larvæ badly disorganized, shrunken and macerated. No ciliary or muscular movement. All dead.
2	80 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 20 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	2.45 P.M.: larvæ shrunken and partially macerated; majority, however, still show slight muscular contractions. A few cases show very feeble ciliary movements. 10 P.M.: all are dead and badly macerated.
3	60 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 40 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	2.50 P.M.: cilia remain active in almost all; in many they suffice for slow swimming movements; slight muscular movements in a majority. Maceration well advanced. 5.35 P.M.: sluggish ciliary and muscular activity in most. 9.45 P.M.; feeble ciliary and muscular activity in most. July 8, 10.00 A.M.: all dead and macerated.
4	40 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 60 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	2.55 P.M.: cilia *more* active, and muscular movements *less* active than in 3 at 2.50. 5.35 P.M.: cilia more active than in 3 at same time; activity enough for slow swimming movements. Muscular movements feeble. July 8, 10.00 A.M.: all dead and macerated.
5	20 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 80 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	3.00 P.M.: larvæ elongated, rigid; less shrunken and with ciliary activity better preserved than in 4 at 2.55; muscular movement almost entirely absent. 5.38 P.M.: cilia active in almost all; a few swim slowly; no muscular movements. 10.00 P.M.: cilia still active in the majority. July 8, 10.00 A.M.: most are dead and macerated; *slight ciliary activity in a few.*
6	10 c.c. $\frac{1}{8}^0$ *n* $CaCl_2$ + 90 c.c. $\frac{1}{8}^0$ *n* $MgCl_2$.	3.03 P.M.: larvæ rigid; cilia active 5.40 P.M.: slight ciliary movements in the majority, though less marked than in 5 at same hour. 10.00 P.M.: slight ciliary movements in most, though feebler than in 5. July 8, 10.15 A.M.: all dead and macerated.
7	100 c. c. $\frac{1}{8}^0$ *n* $MgCl_2$.	3.05 P.M.: ciliary activity almost ceased; persists feebly in a few. No muscular movements. 5.40 P.M.: larvæ all dead and macerated. No ciliary or muscular activity.

result that clumping appears immediately. After an hour's interval most of the cilia have ceased to move, and the larvæ have become disorganized by maceration. Slight muscular contractions persist, however, in many instances. In Solution 2 the immediate effects are very similar, but the initial heliotropic movements are perhaps less marked. Also the solution is less quickly fatal (see Table VI). In Solution 3 we find a much less marked tendency to heliotropism; and in Solution 4 this tendency is no longer evident. Apparently the proportion of Mg has become too high, and the muscular co-ordination requisite for heliotropic orientation is no longer possible. At all events, from here to the end of the series indications of heliotropism are completely wanting. The relatively unfavorable action of Mg on muscular movements as compared with Ca is seen also in the fact that such movements continue for a noticeably shorter period in the solutions containing much Mg. The feeble twitches which in the solutions with much Ca may continue for several hours (see Table VI), are quickly arrested in Solutions 5, 6, and 7, and the more quickly the smaller the proportion of Ca present.

Solutions 3, 4, and 5, it is important to note, are progressively less fatal, the most favorable proportions being those of Solution 5, although Solution 6 is scarcely less favorable. It will be noticed that Mg preponderates over Ca in these solutions just as it does in sea-water; and it will be seen later that the most favorable solutions of the three chlorides, NaCl, $CaCl_2$, and $MgCl_2$ likewise exhibit a large preponderance of Mg. It is certainly a remarkable fact that some vital activities persist for nearly 24 hours in a solution containing no sodium, but merely magnesium and calcium chlorides in certain proportions. This fact, when considered with the quickly fatal effect of a pure solution of either of these salts, affords a further illustration of how necessary it is for normal activity that several different kinds of ions be present in the tissues in definite proportions. The excess of one ion is fatal only in so far as it excludes the presence of the other ions necessary for normal activity. When these are supplied the injurious effect disappears.

VI. Summary.

1. A pure $\frac{3}{4}$ *n* NaCl solution is rapidly destructive of ciliary activity, causing a loss of physical consistency and liquefaction of the ciliary matter. On muscular contractility the injurious action of the NaCl

solution is decidedly less marked. In both cases dilution delays the injurious action.

2. The addition of small quantities of $CaCl_2$ or $MgCl_2$ to a pure NaCl solution much delays its injurious action. The addition of $CaCl_2$ is more favorable for muscular movement; while $MgCl_2$ favors ciliary movement.

3. Identical solutions have different effects on ciliary and on muscular activities. Ciliary movement is capable of continuing for considerable periods in solutions that quickly arrest all muscular activity (*e. g.* of $CaCl_2$, $MgCl_2$, KCl, and mixtures of these salts). In the case of pure $\frac{5}{8}\,n$ NaCl solutions the reverse is true.

4. Solutions that contain no Na-ions quickly deprive the muscles of the power of contractility. Ciliary movements, on the other hand, may continue for many hours in such solutions if favorable proportions of other salts especially $MgCl_2$ and $CaCl_2$ are present.

5. Larvæ that have lost all power of muscular movement but still retain possession of their ciliary activity, no longer show heliotropic orientation.

The effects of solutions containing three chlorides, and the effects following the transference of larvæ from one solution to another, will be described in the concluding section of this paper.

THE EFFECT OF IONS ON THE CONTRACTIONS OF THE LYMPH HEARTS OF THE FROG.

By ANNE MOORE.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

Introduction.

IN a number of recent articles concerning the rhythmical activities of striped muscles, Loeb[1] has reached the following conclusions:

1. Rhythmical contractions occur only in solutions of electrolytes.

2. Ions affecting rhythmical contractions may be divided into three classes: (a) ions which produce rhythmical contractions; of these the most efficacious is Na; (b) ions which retard or inhibit rhythmical contractions, — *e.g.*, Ca and K; (c) ions which act catalytically, that is to say, those which accelerate the action of Na without themselves producing rhythmical contractions directly, — *e.g.*, H and HO.

3. A certain minimal amount of Ca must be present in spite of its antagonistic effect if contractions are to continue for any length of time. If, for example, a Gonionemus is put into a sodium chloride solution of the same concentration as sea-water, its bell contracts rapidly, but very soon ceases to contract. If a little Ca is added to the solution, the contractions are less frequent and last much longer. The addition of Ca lessens the poisonous effects of the sodium. The same point may be illustrated by the effect of such solutions upon young trout. They die in less than forty-eight hours in a pure $\frac{2}{8}n$ NaCl solution, but live eight days in the solution 100 c.c. $\frac{2}{8}n$ NaCl + 8 c.c. $\frac{4}{8}n$ $CaCl_2$.[2]

4. Ions promote rhythmical contractions only because they affect either the physical condition of the colloidal substances, or the rapidity of chemical processes.

Lingle[3] has recently shown that these conclusions drawn by Loeb

[1] Loeb, J.: Festschrift für Fick, 1899, p. 99; This journal, 1900, iii, p. 383; Archiv für die gesammte Physiologie, 1900, lxxx, p. 229.

[2] Moore, Anne: This journal, 1900, iv, p. 386.

[3] Lingle, D. J.: This journal, 1900, iv, p. 265.

from his work on Gonionemus and skeletal muscles, are true for the ventricular muscle of the turtle's heart. Professor Loeb asked me to find out if they were also applicable to the lymph hearts of the frog.

Method.

There are four lymph hearts in the frog, two situated anteriorly, under the scapulae, on a level with the third cervical vertebra; two posteriorly, one on each side of the urostyle. These were removed with as little of the surrounding tissue as possible, and placed in the solutions. To facilitate observation under the dissecting microscope small dishes were used holding about 5 c.c. of the solution the effect of which was to be tested. They were cleaned with potassium bichromate cleaning mixture, washed thoroughly in running water and rinsed in distilled water. Later larger dishes were used, admitting a greater amount of the fluid and an air space. The results obtained were not found to differ from those obtained with the smaller dishes.

The only difficulty encountered was that caused by the presence of parasites, which were especially numerous during the past summer. They often imbedded themselves in the tissue about the hearts, and their movements might easily have been mistaken for the heart-beats. But even when the hearts themselves were not infested, the tissue was so weakened by the parasites that it was not in the best condition for experimental work, and the hearts often failed to respond to favorable solutions. The results from these frogs could not be depended upon; the lymph hearts from such animals are therefore included in the tables in the per cent not beating.

Action of Non-Conductors.

As a rule, in a pure solution of a non-conductor, either no beats took place or the hearts beat feebly and irregularly for a minute or two. Occasionally sustained beating might last eight or ten minutes, but as this might happen in the open air, it could not be ascribed to the presence of the non-conductor, but was probably due to the small amount of serum or lymph remaining in the tissues. Sugar, distilled water, urea, and glycerine were used. Their action may be seen from the following table:

TABLE I.

Solution.	Maximum duration of rhythmical contractions.	Number of hearts used.	Per cent not beating.
Distilled H_2O	2 minutes	13	55
Sugar [1]	8 minutes	8	50
Urea [1]	10 minutes	8	50
Glycerine [1]	1 minute	1	

[1] Approximately isosmotic with $\frac{1}{8}$ *n* NaCl.

Action of Electrolytes.

The effect of electrolytes was very different and very marked. There could be no doubt of their power to initiate and to maintain rhythmical contractions, although the behavior of individual hearts showed considerable variation. In the most favorable cases, strong beats continued with the regularity of the thoracic heart (60 per minute) for hours. The beating then stopped suddenly, or gradually became slower and weaker. In other cases, strong contractions alternated with weak ones or recurred at intervals after a period of rest lasting from a few minutes to many hours. In the latter case it was often possible to excite contractions by stimulating with a glass rod. Occasionally instead of beating as a whole the heart beat in sections, suggesting the fibrillar twitching noted by Loeb in skeletal muscles and by Greene in the turtle's ventricle.

NaCl in pure solution and in combination with $CaCl_2$. — As was to be expected, sodium chloride in a strength approximately isosmotic with the blood was by far the most efficacious of the electrolytes used. In solutions $\frac{2}{8}$ *n* and $\frac{1}{16}$ *n* contractions lasted not longer than fifteen minutes, while in $\frac{1}{8}$ *n* they usually lasted for several hours. It was found that a renewal of the fluid would sometimes cause a heart which had stopped beating to begin to beat again; a fact which suggests the effect obtained by stimulating with a glass rod, as noted above. Lingle has made a similar observation upon the turtle's ventricle. He finds that contractions often become much stronger when the solution is renewed, and that the stimulus of adding a weight often excites contractions which are slow in beginning.

In a few cases the hearts continued to beat in $\frac{1}{8}$ *n* NaCl eight and one-half hours. This was, however, somewhat uncommon and was

probably due to an unusual amount of some antagonistic ion in the tissue. That this conclusion is justifiable may be seen from the effect of a mixture of $CaCl_2$ and NaCl upon a heart which has ceased to beat in a pure solution of NaCl. So soon as too many Na-ions enter the tissue beating is inhibited; neither renewing the solution nor mechanical stimulation will then cause a renewal of the beats; they will instantly be resumed however if the specimen be immersed in a mixture of $CaCl_2$ and NaCl. By alternating the solutions beating may be made to continue indefinitely (Table II). Occasionally a healthy heart does not beat in a pure NaCl solution. In this case it will at once beat in a mixture of NaCl and $CaCl_2$. Owing to the ease with which the tissue is penetrated, the amount of calcium required in the solution is very small, but of course the smaller the amount used, the longer the heart will be in responding to its influence. The fact that a heart exhausted in pure NaCl may be revived marks very strongly the difference between the action of electrolytes and non-conductors. A heart which had ceased to beat in a solution of a non-conductor could never be made to beat again by renewing the solution, by placing it in a solution of another non-conductor, or by mechanical stimulation. If, however, the change was made to NaCl or to a mixture of NaCl and $CaCl_2$ beating was at once resumed.

TABLE II.

Temperature 29°C.

Solution.	Duration of rhythmical contractions.		Number of hearts used.	Per cent not beating.
	Maximum.	Average.		
$\frac{1}{8}$ *n* NaCl	8½ hours	1½ hours	64	25
$\frac{1}{8}$ *n* NaCl alternated with				
100 c.c. $\frac{1}{8}$ *n* NaCl + 4 c.c. $\frac{2}{8}$ *n* $CaCl_2$	10+ hours[1]	3½ hours	74	

[1] + indicates that the heart was left for the night still beating.

As so little calcium is required to prolong the duration of contractions, the addition of K is not necessary to counteract its ill effects, and indeed has been found to be of no advantage. In one case a heart placed in 100 c.c. $\frac{1}{8}$ *n* NaCl + 2 c.c. $\frac{2}{8}$ *n* $CaCl_2$ beat two and one-half hours, while the control placed in the above solution + 2 c.c. $\frac{1}{8}$ *n* KCl beat only one hour.

The experiments upon which the foregoing statements are based were performed during the summer; the temperature at 9.30 A. M. averaged 29° C. and often reached 35° in the middle of the day. In the autumn some of the experiments were repeated and certain variations noted. The hearts were sometimes slower in responding to the solutions, and contractions continued for a much longer time. That these differences were due to the difference in temperature was shown by placing the two anterior hearts of a frog in the same solution, $\frac{1}{8}\,n$ NaCl, one heart being kept at a temperature of 29°, and the other at a temperature of 10°. In six hours the first had ceased to beat, while forty-eight hours later the second was still beating. The average time at 10° was about twenty-four hours, but in one case a heart was beating at the end of sixty-seven hours.

NaCl in combination with SO_4 compounds. — In addition to the use of Ca, the ill effects of a preponderance of Na-ions may be counteracted in another and probably more efficacious way, — by the use of SO_4. Among the SO_4 compounds used were Na_2SO_4, $MgSO_4$, $(NH_4)_2SO_4$, $CaSO_4$, H_2SO_4, K_2SO_4. The last two of these are inhibitory. The first four are distinctly favorable; if from four to ten c.c. of a solution approximately isosmotic with $\frac{1}{8}\,n$ NaCl be added to 100 c.c. $\frac{1}{8}\,n$ NaCl, contractions will be maintained for a considerable time.

TABLE III.

Temperature 29° C.

Solution.	Duration of rhythmical contractions.		Number of hearts used.	Per cent not beating.
	Maximum.	Average.		
100 c.c. $\frac{1}{8}\,n$ NaCl + 4 c.c. $\frac{1}{4}\,n$ Na_2SO_4	9+ hours	3 hours	51	23
100 c.c. $\frac{1}{8}\,n$ NaCl + 4 c.c. $CaSO_4$[1]	8+ hours	6 hours	6	$33\frac{1}{3}$
100 c.c. $\frac{1}{8}\,n$ NaCl + 5 c.c. $\frac{1}{8}\,n$ $MgSO_4$	8+ hours	6 hours	6	$33\frac{1}{3}$
100 c.c. $\frac{1}{8}\,n$ NaCl + 4 c.c. $\frac{1}{10}\,n$ H_2SO_4	$\frac{1}{2}$ hour	$\frac{1}{4}$ hour	6	$66\frac{2}{3}$
100 c.c. $\frac{1}{8}\,n$ NaCl + 4 c.c. $\frac{1}{10}\,n$ K_2SO_4	$\frac{1}{2}$ hour	$\frac{1}{4}$ hour	6	50
100 c.c. $\frac{1}{8}\,n$ NaCl + 4 c.c. $\frac{1}{10}\,n$ $(NH_4)_2SO_4$	$\frac{1}{4}$ hour	$\frac{1}{4}$ hour	6	$66\frac{2}{3}$
100 c.c. $\frac{1}{8}\,n$ NaCl + 2 c.c. $CaSO_4$ + 4 c.c. K_2SO_4	8+ hours	3 hours	6	50

[1] Saturated solution.

At a temperature of 20° C., it was found that in the following solutions,

100 c.c. $\frac{1}{8}$ *n* NaCl + 5 c.c. $CaSO_4$ (sat. sol.),
100 c.c. $\frac{1}{8}$ *n* NaCl + 15 c.c. $\frac{1}{6}$ *n* $MgSO_4$,
100 c.c. $\frac{1}{8}$ *n* NaCl + 5 c.c. $\frac{1}{10}$ *n* $(NH_4)_2SO_4$,
100 c.c. $\frac{1}{8}$ *n* NaCl + 4 c.c. $\frac{1}{4}$ *n* Na_2SO_4,

the maximum length of life was about forty hours.

One may think that the power of Na_2SO_4 to neutralize the ill effects of Na is due to the precipitation of calcium from the tissues. Certain facts, however, are not in accord with this explanation. 1. The addition of $CaSO_4$ to NaCl is almost as efficacious as the addition of Na_2SO_4 in sustaining the contractions of the lymph-hearts. 2. A heart that has been exhausted in $\frac{1}{8}$ *n* NaCl will resume beating if placed either in a solution composed of 100 c.c. $\frac{1}{8}$ *n* NaCl + 4 c.c. $\frac{2}{8}$ *n* $CaCl_2$, or in one composed of 100 c.c. $\frac{1}{8}$ *n* NaCl + 4 c.c. $\frac{1}{4}$ *n* Na_2SO_4; *i. e.*, the same effect seems to be produced by taking away Ca that is produced by adding Ca. 3. A heart that has been exhausted in $\frac{1}{8}$ *n* NaCl and then exhausted in 100 c.c. $\frac{1}{8}$ *n* NaCl + 4 c.c. $\frac{1}{4}$ *n* Na_2SO_4, may be revived in $\frac{1}{8}$ *n* NaCl; *i. e.*, a heart may be revived after precipitating Ca without replacing Ca. It is possible that the Cl ions may have slightly harmful effects which the SO_4 ions are able to neutralize, but, as it has been shown that, as regards rhythmical contraction, the cation is more powerful than the anion, it is more probable that the SO_4 antagonizes Na. The power to neutralize the poisonous effects of Na may be a specific effect of the ions Ca and SO_4, or, as Hardy suggests, it may be merely a function of their bivalency. In the latter case, any bivalent ion should have the same power. Certain experiments were made to test this point, but they were not conclusive. The anterior hearts from one frog were placed, the one in $\frac{1}{8}$ *n* NaCl, the other in one of the following solutions: (temperature 20°).

100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{4}$ *n* Na_2SO_4.
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $CaSO_4$ (sat. sol.).
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{6}$ *n* $MgSO_4$.
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{10}$ *n* Na_2Co_3.
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{10}$ *n* $MgCl_2$.
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{4}$ *n* $CaCl_2$.
100 c.c. $\frac{1}{8}$ *n* NaCl + 4–10 c.c. $\frac{1}{4}$ *n* $BaCl_2$.

The heart in the sodium chloride solution might or might not beat longer than the other. Except in the rather remarkable case where the heart continued to beat sixty-seven hours in $\frac{1}{8}$ *n* NaCl, hearts which

had been exhausted in $\frac{1}{8}n$ NaCl were revived or were made to beat more vigorously by placing them in one of the above solutions.

It will be noted in these solutions that from 4 to 10 c.c. of the electrolyte containing the bivalent ion were sufficient to neutralize the poisonous effects of 100 c.c. $\frac{1}{8}n$ NaCl (the solutions were either equimolecular with $\frac{1}{8}n$ NaCl or they were approximately isosmotic). This proportion speaks in favor of the view advocated by Hardy[1] that the energy of the action of electrolytes upon colloids is a function of their valency; *i. e.*, that 10 cc. of a bivalent metal would be able to antagonize 10^2 or 100 c.c. of a univalent metal.

Other electrolytes. — In addition to the experiments with the chloride of sodium, experiments were also performed with the bromide, iodide, and fluoride. As the Br, I, and F ions are more harmful than the Cl ion, beating stops sooner in these solutions than in the chloride.

Among other electrolytes, $MgCl_2$, NH_4Cl, $CaCl_2$, KCl, and LiCl were used, both alone and with varying proportions of $CaCl_2$. In the first four of these, either no contractions occurred, or they lasted but a few minutes. In LiCl, however, the duration was longer, and when used in combination with $CaCl_2$ this salt maintained contractions three-quarters of an hour. In one case a heart beat seven hours in $\frac{1}{10}n$ $MgCl_2$ (temperature 20°).

TABLE IV.

Temperature 29° C.

Solution.	Duration of rhythmical contractions.		Number of hearts used.	Per cent not beating.
	Maximum.	Average.		
$\frac{1}{8}n$ LiCl	17 minutes	10 minutes	10	40
100 c.c. $\frac{1}{8}n$ LiCl + 4 c.c. $\frac{2}{8}n$ $CaCl_2$	50 minutes	15 minutes	8	$37\frac{1}{2}$
$\frac{1}{8}n$ LiBr	19 minutes	9 minutes	10	30
100 c c. $\frac{1}{8}n$ LiBr + 12 c.c. $\frac{2}{8}n$ $CaCl_2$	36 minutes	15 minutes	6	$33\frac{1}{3}$
100 c.c. $\frac{1}{8}n$ LiBr + 2 c.c. $\frac{2}{8}n$ $CaBr_2$	23 minutes	9 minutes	4	
$\frac{1}{8}n$ NaBr	18 minutes	6 minutes	11	20
100 c.c. $\frac{1}{8}n$ NaBr + 2 c.c. $\frac{2}{8}n$ $CaBr_2$	53 minutes	13 minutes	8	$12\frac{1}{2}$

[1] HARDY, W. B.: Journal of physiology, 1899, xxiv, p. 181.

SUMMARY.

1. The rhythmical contractions of the lymph hearts of the frog depend upon the presence of electrolytes in balanced proportion; for:

(*a*) contractions will not take place in solutions of non-conductors after the salts contained in the serum have been washed out;

(*b*) contractions take place in a pure NaCl solution, but continue longer if a definite proportion of $CaCl_2$ or of some SO_4 compound be added to the solution;

(*c*) a heart which has been exhausted in NaCl may be revived, or may be made to beat more vigorously, if a small amount of the salt of a bivalent ion be added to the solution.

THE EFFECT OF MAXIMUM MUSCULAR EFFORT ON BLOOD-PRESSURE.

By J. H. McCURDY.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

THE teaching of gymnastics, now of such importance in education, can hardly be said to rest upon a sound basis of physiological knowledge. Even the effect of exercise on the blood-pressure, obviously one of the first problems to be considered, has not been determined with sufficient precision. A study of the effect of exercise on the blood-pressure should begin with the selection of one of the forms of exercise in common use by physical trainers. The observations should be repeated upon a number of individuals sufficient to exclude personal idiosyncrasy. Each individual should be observed often enough to make sure that a true record is obtained. Above all, the measurement of arterial pressure should be made during the exercise, as the maximal pressure is not maintained more than a few seconds. For this reason, methods which require the adjustment or manipulation of apparatus during the observation are unsuited to this work. Measurements after the muscular effort do not show the blood-pressure during the effort. So far as I am aware, none of the investigations hitherto made in this field satisfies these indispensable conditions.

Types of exercise. — Teachers of gymnastics recognize certain well defined types of muscular exercise.

"Exercises of speed" are those in which the individual movements follow each other with great rapidity. Each individual effort is necessarily far less than the maximum effort possible to the group of muscles concerned. Exercises of speed may be divided into those of local character, for example, piano playing, and those of general nature, as sprinting.

"Exercises of endurance" are characterized by long-continued moderate endeavor. They also may be local, as in the file-cutter's trade, where the muscles of the arm and shoulder are used almost continuously, or they may be general, as in mountain climbing or

long-distance swimming, which require the relatively moderate contraction of large groups of muscles during long periods.

"Exercises of strength, or effort" demand great muscular exertion for a very brief period. In these the glottis is closed at full inspiration and the chest walls fixed, in order to give a suitable support to the muscles of the trunk. Good examples of such exercises are wrestling and the lifting of heavy weights.

Previous writers have shown that not too vigorous general exercise, *i. e.*, a mixture of several kinds of exercise, will raise the blood-pressure. Knowledge of the changes in blood-pressure and other functions during each of the different kinds of exercise is now required. The present investigation deals with the alterations in blood-pressure during "exercises of strength or effort."

Method. — It was thought better to select one clearly defined, typical exercise of strength, rather than to attempt the study of the blood-pressure in a variety of movements. The exercise selected was a combination of the back and leg lift used in the physical examination of most college students. The subject stood with bent knees. With the left hand he grasped the middle of the dynamometer handle, either end of which rested on the front of the thigh. At the word, he extended his legs and straightened his back, with all his strength. From three to five lifts were necessary to determine the highest blood-pressure.

The blood-pressure was measured with the sphygmomanometer. The instrument employed in this research was a modification of that used by Hill and Riva-Rocci. It rests in principle upon the fact that the pulse may be obliterated in any artery not possessing too free anastomoses by subjecting the artery to an external pressure equal to the blood-pressure in the artery at the point of compression added to that necessary to compress the tissue surrounding the artery.

A hollow arm tube of thin, flaccid rubber, 4 cm. wide, and 7 mm. inside diameter, was made from the inner tube of the tire of a racing bicycle. The interior of the tube communicated by means of a bicycle valve stem with a pressure bottle suspended from a hook 5 metres above the floor. The arm tube was covered by an inelastic casing of leather (raw-hide) 36 cm. long and 9 cm. wide. Ordinary shoe hooks were fastened to the leather, so that the band could be brought closely round the smallest arm and laced into position as a shoe is laced. The arm tube was applied closely and evenly to the arm, with the upper border touching the beginning of the deltoid enlargement. It

remained in position without being bound tightly enough to interfere with the venous circulation. The tube leading to the pressure bottle was clamped near its connection with the arm tube and the pressure bottle then raised to a height sufficient to obliterate at once the pulse in the arteries distal to the arm tube. The pressure usually made was 200 cm. of water. The clamp was now quickly removed, and the bottle lowered until the radial pulse, which had disappeared when the pressure was made, was perceived to be just returning. During the last part of inspiration and the first part of expiration, the pulse returned at somewhat higher pressure than at other times. The number of heart-beats which could be counted during the muscular effort before the pulse disappeared varied from one to four. The pressure fell in a few seconds after the beginning of the lift because the subject could not longer maintain his maximum muscular effort, *i. e.*, as the tension of the dynamometer spring decreased and the amount of work accomplished by the subject grew less the arterial pressure fell. This made it necessary to lower the pressure bottle to record a new pressure level.

Sources of error. — When the sphygmomanometer is applied to any artery force must be employed to compress the tissues surrounding the artery. In the case of the radial artery Von Basch estimates the force required at 6 to 8 mm. Hg. The occlusion of the empty artery requires a pressure of from 1 to 5 mm. Hg. He calculates that changes in the relation of the radial artery to the bone and tendons caused by accidental movements of the hand or fingers may introduce an error of 20 to 60 mm. Hg. Finally, the error of observation in determining when the artery is actually occluded may amount to from 1 to 5 mm. Hg. Some of these errors may fairly be excluded when Hill's instrument is applied to the upper arm. In this position the relation of the artery to surrounding parts does not change materially. It would then seem fair to limit the errors to the occlusion of the artery, the compression of the surrounding tissues, and the errors of observation. These would make a total of from 8 to 18 mm. Hg. To this would be added the pressure lost in stretching the tube encircling the arm, but the friction here is so great, that for all practical purposes the tube is inextensible, and the error from this source may therefore be neglected. Hill affirms that when the tube is placed around the upper arm the only material error is one of observation, and he limits that to 5 mm. Hg. Hill finds in experiments on dogs that when the tube is placed around the neck, the readings are iden-

tical with those taken simultaneously from the femoral artery connected directly with a mercury manometer. We may believe, then, that the readings taken in the present investigation are at the most not more than 20 mm. Hg greater than the absolute blood-pressure, and are probably much nearer.

Changes in blood-pressure. — The blood-pressure was recorded in seventy-seven experiments on twenty-three men before, during, and two to three minutes after the maximum lift. The pulse rate was recorded before and after the lift. The average of all the measurements was as follows: —

	Subject standing.	Subject lying.
Blood-pressure in mm. of mercury before lift . .	111	110
Blood-pressure in mm. of mercury during lift . .	180	
Blood-pressure in mm. of mercury two to three minutes after lift	110	110

It appears that the blood-pressure undergoes a sudden and great increase during the lift, and that it falls very rapidly to normal as soon as the muscular effort ceases. This rise in blood-pressure is not accompanied by any great change in the pulse rate. In seventeen examinations on nine individuals during the five seconds of the lift and the fifteen seconds immediately following, seven showed an average increase of five beats per minute, seven showed a decrease of four beats and three continued at the same rate. The pulse rate in these cases returned to normal one minute after the lift.

The rapid return of blood-pressure and pulse rate to normal after the maximum exercise of strength is in marked contrast to the slow return observed after exercises of speed. Hill[1] gives the pulse rate of an individual sitting quietly as 64, the arterial pressure as 98 mm. Hg; after running rapidly 400 yards, the pulse of this individual rose to 100, and the blood-pressure to 120–130 mm. Hg. Ten minutes later the pulse was still 100 and the blood-pressure 110–115 mm. After a rest of one hour and twenty minutes the pressure had fallen slightly below normal (90–95 mm. Hg); the pulse, however, still continued sixteen beats above normal. The present study shows that in lifting both blood-pressure and pulse rate return to the normal level within three minutes after the lift. This difference between exercises of effort and exercises of speed is of importance. Exercises of maximum strength subject the heart and blood-vessels to great and sudden strain, while exercises of speed as a rule encourage functional activity without an immoderate increase in blood-pressure.

[1] Hill: Journal of physiology, 1897–98, xxii, p. xix.

TABLE I.

The blood-pressure in eleven men before, during, and after lifting with maximum strength; the pulse rate[1] before and after the lift. Average of several observations made on each individual.

Name.	Age.	Weight in kilos.	Weight lifted in kilos.	Before lift.		During lift.	2–3 minutes after lift.		No. of beats in distal artery between compression and disappearance of pulse.	Condition of arm.
				Pulse rate.	Blood-pressure, mm. Hg.	Blood-pressure.	Pulse rate.	Blood-pressure.		
Sk.	31	70	216	69	109	210	74	113	1–4	Moderate size, muscular.
Ma.	23	61	118	93	109	165	94	107	1 4	Small, not muscular.
De.	30	60	131	75	93	146	76	95	1	Small, not muscular.
Sa.	31	68	188	67	100	175	67	101	1–2	Large, muscular.
McC.	33	75	170	78	124	178	81	125	1–2	Moderate size, muscular.
Ar.	21	75	178	77	117	207	80	117	1–3	Large, muscular.
St.	41	83	149	78	122	202	77	114	1–3	Large, muscular.
Hi.	25	60	155	84	100	154	80	107	1–2	Small, not muscular.
Be.	26	61	133	77	108	157	78	108	1	Moderate size, muscular.
Me.	27	72	249	76	107	188	78	110	1–4	Large, muscular.
Ja.	26	75	152	73	127	197	74	130	1–2	Moderate size, muscular.

[1] The pulse rate was recorded in the recumbent as well as the standing position. It seemed necessary to give here only the figures for the standing position. The glottis was closed during each lift.

TABLE II.

The blood-pressure in four men before, during, and after lifting with maximum strength; the pulse rate[1] before and after the lift. Every observation made is recorded.

Name.	Age.	Weight in kilos.	Weight lifted in kilos.	Before lift.		During lift.	2–3 minutes after lift.		No. of beats in distal artery between compression and disappearance of pulse.	Condition of arm.
				Pulse rate.	Blood-pressure, mm. Hg.	Blood-pressure.	Pulse rate.	Blood-pressure.		
Sk.	31	70	225	64	114	195	75	118	1	Moderate size, muscular.
			220	72	114	206	72	114	3	
			210	72	108	199	72	105	3	
			170	72	104	199	78	118	1	
			223	70	107	199	70	107	1	
			245	66	105	265	78	114	1	
Sa.	31	68	150	62	88	125	54	96	1	Large, muscular.[2]
			190	66	125	221	60	103	1	
			200	72	103	184	72	103	1	
			195	66	97	140	66	96	1	
			220	66	84	184	69	94	1	
			170	72	104	199	78	118	2	

De.	30	60	100	72	92	154	72	92	1	Small, not muscular.
			135	70	88	147	70	98	1	
			60	72	90	140	72	85	1	
			160	84	92	132	90	98	1	
			140	72	97	169	72	92	1	
			155	78	98	132	78	107	1	
Ma.	23	61	60	100	110	125	96	99	1	Small, not muscular.
			90	96	107	136	106	110	1	
			110	93	107	162	96	107	1	
			170	88	114	191	90	114	1	
			160	90	105	213	84	107	3	

[1] The pulse rate was recorded in the recumbent as well as the standing position. It seemed necessary to give here only the figures for the standing position. The glottis was closed during each lift.

[2] Subject was tired from cycling.

In Table I, page 99, are shown the blood-pressure in eleven men, before, during, and after the maximum lift, and the pulse rate before and after the lift. In each instance the figures given are the average of several observations made on each individual. In Table II, pages 100, 101, all the observations made on four individuals are recorded, in order that the influence of maximum efforts made at different times by the same individual may be studied. The essential facts brought out by these tables are expressed in the averages already discussed. The pulse rate was recorded in the recumbent as well as the erect position, but it seems necessary to give only the figures for the latter.

Changes in intra-pulmonic and intra-abdominal pressure. — It is *a priori* somewhat probable that the sudden great rise of blood-pressure on maximum muscular effort is due in large part to an increase in the intra-pulmonic and intra-abdominal pressure.

To determine this question experiments were made on the rise in blood-pressure produced by expiratory efforts with closed glottis. As a rule there was seen an increase in blood-pressure somewhat greater than that observed during the lift by the same individual (Table III).

TABLE III.

Blood-pressure in millimetres of mercury before and during forced expiratory movements with closed glottis.

Individual.	Before.	During.	Average increase.
Sk.	106	216	120
Sa.	96	201	105
McC.	121	199	77

Evidently there is a marked rise of blood-pressure during forced expiratory movements with closed glottis. The question now arises, What relation does this rise in blood-pressure bear to increased intra-pulmonic and intra-abdominal pressure? The intra-pulmonic pressure was determined approximately by ascertaining how high a column of mercury the subject could support by an expiratory effort continued for five seconds, *i. e.*, the time of an average lift. The following figures, 62, 60, 58, 60, 60, 58, 64, show that the average increase in intra-pulmonic pressure in a typical case was 60.3 mm. Hg.

The intra-abdominal pressure during forced expiration with closed glottis was also studied. An ordinary stomach tube was introduced into the stomach and connected with a mercury manometer level with the stomach. Half a litre of water was placed in the otherwise empty stomach, and the connecting tubes all filled with water containing no air bubbles. The subject then made a vigorous expiratory effort with the glottis closed, and the pressure which could be maintained during the average period of a maximum lift was recorded. The following observations, 84, 58, 102, 108, 50, 120, on one individual show an average increase in intra-abdominal pressure of 87 mm. Hg.

It has already been mentioned that the average increase in intra-

pulmonic pressure during expiratory efforts was 60.3 mm. Hg. The average increase in blood-pressure during expiratory efforts was 77 mm. Hg. (Table III, average of observations on McC.). It seems probable, therefore, that the increase in blood-pressure during lifting with closed glottis is due in large measure to the increase in intra-pulmonic and intra-abdominal pressure. The increase in intra-abdominal pressure in one individual while lifting is given in the following figures, 70, 90, 70, 64, 92, 80; average, 77.6 mm. Hg. This average increase of pressure within the abdomen during lifting (77.6 mm. Hg) should be compared with the average increase in blood-pressure during lifting in the same individual (53.8 mm. Hg, Table I, average of observations on McC.). It will appear that the increase in intra-abdominal pressure during lifting is as great or greater than the increase in blood-pressure.

Conclusions.

1. A true record of the changes in blood-pressure in consequence of maximum muscular effort is obtained only when the record of blood-pressure is taken during the effort. The blood-pressure falls too rapidly to make later observations trustworthy.

2. The blood-pressure during "exercises of maximum effort" (maximum lift) rises suddenly to a great height. The frequency of the heart beat is very slightly changed. At the conclusion of the maximum effort, which can be maintained but a few seconds, the blood-pressure and the frequency of the heart beat very rapidly return to normal. There is in this respect an important difference between "exercises of effort" and "exercises of speed."

3. Maximum exercises of strength increase the intra-pulmonic and intra-abdominal pressure as much or more than they increase the blood-pressure. It seems probable that the increase in blood-pressure is due largely to this rise of pressure in the abdomen and thorax.[1]

[1] I am indebted for much assistance to Dr. W. Muhlberg, Mr. N. E. Sanders, and Mr. W. Skarstrom; and I am also greatly obliged to the twenty-three men who gave their services as subjects of observation.

PHYSIOLOGICAL AND TOXICOLOGICAL EFFECTS OF TELLURIUM COMPOUNDS, WITH A SPECIAL STUDY OF THEIR INFLUENCE ON NUTRITION.[1]

By L. D. MEAD and WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry of Columbia University, at the College of Physicians and Surgeons, New York.*]

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ABOUT two years ago Professor Victor Lenher was engaged at this University with extended studies of the properties of tellurium and its compounds.[2] The ill effects which Professor Lenher experienced from involuntary inhalations of volatile products formed in preparing tellurium impressed him with the desirability of a systematic study of its physiological effects. He generously offered to furnish Dr. Gies with pure tellurium preparations for such an investigation. We wish to thank Professor Lenher for the suggestion

[1] A preliminary account of some of the experiments referred to in this paper was given in abstract in the Proceedings of the American Physiological Society. This journal, 1900, iii, p. xx.

[2] See Journal of the American Chemical Society, 1899, xxi, p. 347; 1900, xxii, pp. 28, 136.

which led to these experiments, and for the costly material without which they would not have been possible. We are also greatly indebted for valuable facts communicated by Professor Lenher from his large chemical experience.

I. Influence on Metabolism.

With the exception of a brief and very imperfect experiment by Beyer (13),[1] on the excretion of urea after intravenous injection of sodium tellurate, no special study has ever been made of the influence of compounds of tellurium on the nutritional processes in the body.[2] Neusser (6) was the first to note that potassium tellurate induces anidrosis. In about fifty clinical experiments, on as many consumptives, he observed that the night-sweats were very perceptibly reduced after administrations of potassium tellurate in daily doses of 0.02–0.06 gm. Subsequent investigators, principally Pohorecki (7), Combemale and Dubiquet (8) and Czapek and Weil (10) confirmed this observation of the physiological action of tellurates, and Combemale (9) even expressed the conviction that sodium tellurate is one of the very best antisudorific agents. Consequently, both potassium and sodium tellurates have been employed for the purpose of arresting sweating, particularly the colliquative sweats of phthisis.[3] Further, tellurium is repeatedly found, in small quan-

[1] The numerals in parentheses correspond with those preceding the references in chronological arrangement at the end of this paper.

[2] Tellurium was discovered in 1782 by Müller von Reichenstein and identified and named (*tellus*, the earth) by Klaproth in 1798. The metal is silver-white, of markedly crystalline structure, and possesses a strong metallic lustre. Its atomic weight is still uncertain, but closely approximates 128. (See note, page 148.) Tellurium is very nearly related chemically to sulphur and selenium. Its chemical qualities have offered difficulties from the time of its discovery, so that at first it was called *aurum paradoxum* and *metallum problematicum*. It is one of the rarer elements and occurs in nature mostly as telluride in combination with bismuth, lead, mercury, silver, and gold. The following formulæ show the composition and relationships of the tellurium compounds referred to in this paper:

Tellurous oxide, TeO_2.	Sodium tellurate, Na_2TeO_4.
Telluric oxide, TeO_3.	Hydrogen telluride, H_2Te.
Tellurous acid, H_2TeO_3.	Tellurium tartrate, $Te(C_4H_5O_6)_4$.
Telluric acid, H_2TeO_4.	Methyl telluride $(CH_3)_2Te$.
Sodium tellurite, Na_2TeO_3.	Ethyl telluride $(C_2H_5)_2Te$.

[3] Cerna: Notes on the newer remedies, 2d ed., 1895, pp. 164 and 185. See also, New York medical journal, 1891, liii, p. 370, on camphoric acid and tellurate of sodium as anidrotics, referring to the recommendations in La province médi-

tity, in commercial bismuth preparations,[1] and their medicinal use implies frequent incidental action of this tellurium impurity. In view of these facts, we have attempted first of all in our experiments to determine the influence of small non-toxic quantities of tellurium on metabolism, as measured especially by fluctuations in the excretion of nitrogen.

Conduct of the Experiments.

Animals and Environment. — The experiments were performed on full-grown dogs weighing from 10 to 16 kilos. The general methods were those outlined in the report of some previous investigations made by Dr. Gies under Professor Chittenden's supervision.[2] The animals were confined in a suitable cage, well adapted for the collection and separation of fluid and solid excreta. The cage was open at the top so as to permit of free circulation of air, and was kept in a comfortable room with a constant temperature.

Character of Diet. Feeding. — The animals received regularly a mixed diet of hashed lean meat, cracker dust, lard, and water. Former experience proved this to be a very acceptable, digestible and nutritious mixture. The hashed meat was prepared by a method similar, in general, to that previously described by Dr. Gies.[3] The hash was preserved *frozen* with results which were satisfactory throughout all the experiments. Commercial cracker dust, containing only 1.51 per cent of nitrogen, afforded the carbohydrate element of the diet. This was kept entirely dry in large quantity in well stoppered bottles. The lard employed was perfectly fresh. Ordinary river water was used. Neither lard nor water contained appreciable quantities of nitrogen.

The daily mixed diet was given regularly in two equal portions, in the morning at nine and in the evening at six o'clock. The water was stirred with the other ingredients, until the whole mixture had the consistency of very thick soup. This mixture, while not very appetizing in appearance, possessed an agreeable odor and was always lapped eagerly by all the animals in the normal periods. The food

cale. Tellurates have not, however, come into general employment, because of the obnoxious odor imparted to the breath after their administration. See page 130.

[1] See Ekin and Brownen: American journal of pharmacy, 1876, xlviii, p. 133 (Abstr.). Blyth: Poisons, their effects and detection, 1885, p. 559. Janzon: Druggists' circular and chemical gazette, 1894, xxxviii, p. 256 (Abstr.).

[2] Chittenden and Gies: This journal, 1898, i, p. 4. [3] *Ibid.*, p. 5.

was presented in a common glass crystallization dish, a receptacle especially suited for the licking up of last traces.

Dosage, Weighing, etc. — The daily doses of tellurium were also divided equally. Each half was enclosed in a capsule made of a small portion of the weighed hash. This was always quickly swallowed, in eager anticipation of the rest of the meal, which followed immediately, so that the tellurium entered the stomach almost simultaneously with the main portion of the food.

In the records of the experiments each period of twenty-four hours ended at 9 o'clock in the morning, when the first food of the new day was given. The animal was weighed just before that hour. The daily analytic data are for the twenty-four hours ending at 9 A. M. The figures representing weight are therefore for the weight at the *end* of each experimental day.

Collection of Excreta. — It was found in the experiments already alluded to[1] that diurnal variations in the elimination of urine were practically neutralized at the end of a week or ten days. Consequently, in these experiments, in which the periods were of from seven to ten days' duration, it was unnecessary to remove any urine with a catheter. We collected the urine as it was excreted naturally and thus avoided the disturbances which may arise from catheterization. At the end of each day the interior of the cage was thoroughly sprayed, and rubbed with a stiff test-tube brush. After the physical qualities of the combined 24 hours' urine had been noted, the cage washings were used in making up the daily volume of urine to a litre, in preparation for analysis. Powdered thymol was added in order to prevent bacterial changes. This was at times particularly desirable, for not all the analyses could be begun on the day of collection.[2]

No special indigestible substance was introduced with the food to mark off the fæces. As the elimination of solid excreta from the dog is quite regular under normal conditions, and also when equilibrium is maintained, it seemed best to refer the excrementitious matter from the intestines to the period of their collection. While this course permits of error, only unimportant influences on character and elimination would be hidden under these conditions. The

[1] CHITTENDEN and GIES: This journal, 1898, i, p. 4.

[2] Some of this urine remained in the laboratory for almost two years, without undergoing any change in nitrogen content. A very thin scum formed during that time and the urine became a little darker in color.

inaccuracies of deduction resulting from this procedure certainly could not have been material in our work, since the figures for the nitrogen in the fæces of whole periods, to be given farther on, are essentially the same for each period in a group.[1] The fæces were thoroughly desiccated over the water bath on a weighed dish immediately after collection, then weighed, thoroughly ground, preserved in dry, well-stoppered bottles, and analyzed at convenient intervals.

An appreciable quantity of hair falls from most dogs during such an experiment. This was collected daily, combined for each period, and the nitrogen content determined. It will be observed, in the tables giving analytic data, that the nitrogen thrown off in this way is so considerable that it must be taken into account in equilibrium experiments. From long-haired dogs the loss of hair is especially marked. The nitrogen eliminated in this way is not the same for each period, as our results will show.[2]

Analytic methods. — Nitrogen of the food and excreta was determined by the Kjeldahl process, in all except the last experiment. Oxidation was accomplished with sulphuric acid aided by copper sulphate.[3] In the urine of the last experiment nitrogen was estimated by the hypobromite method with Marshall's apparatus.[4] Urea was calculated from the nitrogen thus obtained (1 c.c. N = 0.00282 gm. urea). Total sulphur and phosphorus were determined by the usual fusion methods;[5] phosphoric acid by Mercier's modification of Neubauer's method;[6] total and combined sulphuric acid gravimetrically by customary methods, the former with Salkowski's precaution,[7] the latter by Baumann's process;[8] uric acid by Ludwig's well-known silver method;[9] fat (ether-soluble matter) in the fæces by extraction with anhydrous ether in the Soxhlet apparatus in the usual manner. The total solids in the urine were calculated from the volume and the specific gravity ("Christison's formula") with the aid of Haeser's coefficient.[10] Indoxyl was estimated quali-

[1] See tables giving quantitative elimination of fæces, composition, etc., under similar conditions: CHITTENDEN and GIES, *loc. cit.*, p. 37.

[2] See also, *Ibid.*, pp. 24 and 33.

[3] MARCUSE: Archiv für die gesammte Physiologie, 1896, lxiv, p. 232.

[4] MARSHALL: Zeitschrift für physiologische Chemie, 1887, xi, p. 179.

[5] Given in detail by CHITTENDEN and GIES: *loc. cit.*, page 7.

[6] NEUBAUER and VOGEL: Analyse des Harns, zehnte Auflage, 1898, p. 731.

[7] *Ibid.*, p. 721.

[8] *Ibid.*, p. 724.

[9] *Ibid.* p. 820.

[10] *Ibid.*, p. 703.

tatively with the Jaffe-Stokvis test.[1] The specific gravity of the urine was ascertained with the ordinary urinometer. The reaction to litmus was taken. When the urine was amphoteric, the stronger reaction was recorded. The quantities of which analyses were made were those customarily employed.

Tellurium was determined quantitatively in the following manner: Solid excreta, after fine division in a mortar, and also concentrated urine, were treated with strong hydrochloric acid and potassium chlorate over the water bath until completely disintegrated and almost perfectly dissolved. After that had been accomplished the fluid was kept on the bath until it was entirely freed of chlorine gas. It was then concentrated to 400-500 c.c. and filtered. The clear acid filtrate was next saturated, while warm, with sulphur dioxide gas and allowed to stand for 24 hours. The bluish black metallic tellurium which had separated in this process was then filtered on a weighed paper, washed with dilute acid, dried at 110° C to constant weight, and determined gravimetrically.[2]

First Experiment; with Tellurous Oxide.

The animal used in this experiment was a long-haired bitch weighing approximately 15 kilos. A preliminary period of six days sufficed to bring her into nitrogenous equilibrium. The daily diet throughout the experiment was 250 gms. of prepared meat (9.099 gms. N), 50 gms. of cracker dust (0.755 gm. N), 40 gms. of lard, and 700 c.c. of water, containing a total of 9.854 gms. of nitrogen. The experiment continued twenty-four days, and was divided into three periods: a fore period of seven days during which normal conditions prevailed; a longer period of ten days during which doses of tellurous oxide, averaging nearly 0.1 gm., were given twice daily; and an after period, equal in length to the first, during which no tellurium was administered. During the tellurous oxide period of ten days a total of 1.6 gm. of the oxide was retained after ingestion, or 0.16 gm. per day. The smallest dose was 0.05 gm. in half of the food for the day; the largest was 0.5 gm. in the same quantity of food.[3]

[1] Neubauer und Vogel: Analyse des Harns, zehnte Auflage, 1898, p. 166.

[2] This method, Professor Lenher assures us, gives accurate quantitative results. The methods employed by Hansen, Kletzinsky and Hofmeister were much the same. Kletzinsky: Wiener medicinische Wochenschrift, 1858, viii, p. 355.

[3] The daily dose of tellurate, in therapeutic use, recommended by Neusser, Pohorecki and Combemale and Dubiquet, varies from 0.01 to 0.06 gm.

On the first day two doses of 0.25 gm. were given. A few minutes after the first dose was administered, the characteristic alliaceous odor became quite noticeable in the expired air and it increased steadily during the rest of the day. On the following morning the odor in the room was of sickening intensity. No special change except languor and sleepiness had been noticed in the animal itself up to this point. The dose in the morning meal (second day) was raised to 0.5 gm. But this was clearly a mistake, for, although the food with its contained tellurium oxide was eaten eagerly and quickly, the whole meal was vomited in less than ten minutes afterward.[1] The vomit was collected quantitatively. The evening portion of food contained only 0.25 gm. The dog ate it very slowly, but before swallowing all of it, vomited violently what had just been eaten. This vomited material was also gathered quantitatively and added to that collected in the morning. The uneaten portion of the evening meal was mixed with the vomit of the day, and the whole thoroughly desiccated on the water bath for determination of its nitrogen content, which was found to be practically equivalent to that of the day's food.[2] The dog was sick throughout the second day. The urine, 220 c.c., was coffee colored.[3] It contained no granular tellurium, although some was held in solution. Bile pigment, albumin, and sugar were also absent.

At this stage of the experiment it was obvious that the animal had been thrown completely out of physiological equilibrium.[4] The quickest way to restore the equilibrium seemed to be to feed the dog an extra amount of food equivalent to the previous day's meal.

[1] In a few preliminary experiments on two other dogs of about the same size it was found that 0.75 to 1.0 gm. of the oxide administered in the same manner caused vomiting, but that 0.5 gm. did not. We had hoped, therefore, that this dose would be safely ingested, at least once, so that we should be able to determine very definitely what metabolic influence tellurium might exert under conditions approximately toxic: and yet not toxic enough to vitiate the experiment. It will be seen that this was practically accomplished, in the case of this particular dog.

[2] It contained 10.335 gm. of nitrogen. The food contained 9.854 gm. The difference (0.481 gm.) was doubtless due to the nitrogen of the mucus, etc., thrown from the stomach.

[3] Somewhat darker than No. 8 in Vogel's well known scale of urine tints. See Tyson: A guide to the practical examination of urine, 1806, 9th ed., frontispiece.

[4] The dosage period was lengthened to ten days on account of this occurrence. See Chittenden and Gies, *loc. cit.*, page 9, for an account of similar experiences, with favorable outcome.

This amount was given in two equal portions on the third day, with the gratifying results shown in the tables for this experiment. Although cumulative action of the tellurium had been manifested, the dog's appetite did not seem to be at all impaired at this time. The food on the third day contained 0.25 gm. of the oxide. During the remaining seven days of the oxide period, the dosage was kept as high as was deemed expedient. On the evening of the fourth day the animal was again nauseated, although the food with its dose of 0.125 gm. of tellurous oxide was finally eaten and none thereafter vomited. For the rest of the period the daily amount—0.1 gm.—gave no special trouble. The dog was very stupid on the third and fourth days of the dosage period, and manifested a constant tendency to sleep. On the fifth day it was more lively and toward the end of the period was entirely normal. At the close of the experiment 0.5 gm. of the oxide in the usual quantity of food induced vomiting within an hour.

The color of the urine throughout the tellurous oxide period was considerably darker than normal, but this difference was less and less perceptible after the day of the greatest dosage. Only now and then could the odor of methyl telluride be detected. Indican was present in samples of each period. Bile pigment, sugar, coagulable proteid and abnormal sedimentary material were absent. Tellurium in small quantity could be detected in the urine during the first half of the period. The fæces were not greatly changed; they were somewhat more bulky, contained more mucus, and were bluish-black instead of brown, as in the fore period, and late in the after period. Occasionally the odor of methyl telluride in the fresh fæces was recognized, though usually it was lost in that of the normal fæcal aromatic compounds. The alliaceous odor in the dog's breath was most marked at about the middle of the experiment, when it began to diminish, although, so long as the animal remained under observation — for almost five weeks after the last dosage — it was very marked. The shed hair gave off distinctly the odor of the methyl compound, yet we were unable to separate any tellurium from it.

The accompanying tables, pp. 112 and 113, give the various analytical results and other data of the first experiment.[1]

[1] The first three metabolism experiments were performed before Mr. MEAD had been invited to assist in this research, and during the year when the routine labor connected with the equipment of the Department of Physiological Chemistry and its organization for regular work was most exacting. Hence it was im-

FIRST EXPERIMENT.

Fore Period.

Date 1898 Dec.	Body weight. K.	Food. Nitrogen. Grams.	TeO_2. Gms	Urine. Vol. c.c.	Sp. gr.	Reaction litmus.	Nitrogen. Grams.	Phosphorus. Grams.	Sulphur. Grams.	Uric acid. Grams.	Fæces. Dry weight. Grams.	Nitrogen. Grams.	Ether-soluble matter. Grams.	%
1	14.9	9.854	..	578	1017	Acid	10.421							
2	14.8	9.854	..	590	1016	Acid	10.013	1.321			10.57	0.698	2.915	27.6
3	14.9	9.854	..	782	1013	Acid	9.403		1.794					
4	15.0	9.854	..	651	1013	Acid	8.921			0.242				
5	14.8	9.854	..	720	1013	Acid	9.410	2.005			21.82			
6	15.0	9.854	..	665	1014	Acid	8.960					1.676	9.711	26.9
7	15.0	9.854	..	639	1012	Acid	8.231	1.198	2.529	0.199	14.28			

Tellurous Oxide Period.

8	15.0	9.854	0.50	711	1013	Acid	8.982							
9	14.3	(9.854)	(0.75)	220	1035	Alkaline	3.654	0.982						
10	15.1	19.708	0.25	865	1014	Acid	13.117		1.601		22.14			
11	15.0	9.854	0.25	731	1014	Acid	10.031			0.284		2.692	13.332	33.8
12	14.9	9.854	0.10	795	1015	Acid	12.831	2.334			17.28			

13	15.0	9.854	0.10	650	1016	Acid	9.992							
14	15.0	9.854	0.10	709	1014	Acid	9.674		2.834					
15	15.1	9.854	0.10	640	1015	Acid	8.361				28.96			
16	15.0	9.854	0.10	690	1014	Acid	8.904					2.462	13.976	33.4
17	15.1	9.854	0.10	704	1013	Acid	9.206	3.368	1.842	0.328	12.86			
After Period.														
18	15.0	9.854	..	692	1014	Acid	9.002							
19	14.9	9.854	..	708	1013	Acid	9.434		1.196		21.38			
20	15.0	9.854	..	660	1014	Acid	8.831	1.892				1.897	12.320	32.8
21	15.1	9.854	..	670	1015	Acid	9.324		1.219		16.21			
22	15.0	9.854	..	715	1013	Acid	9.238							
23	15.1	9.854	..	635	1015	Acid	8.597							
24	15.1	9.854	..	693	1014	Acid	8.768	2.805	1.821	0.468	26.74	1.394	7.790	29.1
Daily Averages for each of the Three Periods.														
Fore period (7 days)		9.854	..	661	..		9.337	0.646	0.617	0.063	6.66	0.339	1.804	27.1
Tell. oxide period (10 da.)		9.854	0.16	672	..		9.475	0.668	0.628	0.061	8.12	0.515	2.731	33.6
After period (7 days)		9.854	..	682	..		9.028	0.671	0.605	0.067	9.19	0.470	2.870	30.1

The tables show at a glance that during this experiment tellurium had no material influence on the weight of the animal, that the volume and reaction and specific gravity of the urine were not particularly altered, that the quantities of phosphorus, sulphur and uric acid excreted were uniformly the same, and that the nitrogen elimination was but little affected. The following summary gives the quantitative and the percentage distribution of nitrogen for each period:

Total nitrogen.	Fore period. Grams.		Tellurous oxide period. Grams.		After period. Grams.	
Nitrogen of food	68.978		98.059[1]		68.978	
Nitrogen of urine	65.359		94.752		63.194	
Nitrogen of fæces	2.374	68.787	5.154	101.138	3.291	67.669
Nitrogen of hair	1.054		1.232		1.184	
Nitrogen balance	+ 0.191		− 3.079		+ 1.309	
Ratio to nitrogen ingested.	**Per cent.**		**Per cent.**		**Per cent.**	
Nitrogen of urine	94.8		96.6		91.6	
Nitrogen of fæces	3.4		5.3		4.8	
Nitrogen of hair	1.5		1.3		1.7	
Nitrogen balance	+ 0.3		− 3.2		+ 1.9	

[1] Quantity remaining after subtraction of the nitrogen of the vomit. See footnote, p. 110.

possible to make daily detailed analyses of each 24 hours' urine, and Dr. GIES had to be content, in some cases, with results obtained from the urine of several days combined. The totals and daily averages were, of course, in no wise affected by this. Thus, throughout the three periods of the first experiment, the data for phosphorus, sulphur, and uric acid are for urine passed during several days. The figures are recorded on the last day of each separate combination. The dry weight of the fæces is recorded on the days of elimination. The 0.75 gm. of tellurous oxide given on Dec. 9 is not included in the total for the period (1.6 gm.), because practically all of it was ejected in the vomit. See pages 110 and 111 for references to the latter and the variations in quantity of food on Dec. 9 and 10. The average daily weight of hair shed was 1.24 gm. in the fore period (0.150 gm. N), 0.99 gm. in the tellurous oxide period (0.123 gm. N), and 1.35 gm. in the after period (0.169 gm. N).

These results show that in spite of the relatively large doses of tellurous oxide (quantities greater than therapeutic doses for man), given repeatedly during a period of ten days, the animal remained approximately in nutritive equilibrium. They also show that the immediate ingestion of food equal to that vomited, sufficed to restore promptly the balance that had been disturbed on the second day of the oxide period. It should, of course, be remembered, in considering the effect of quantity in this connection, that tellurous oxide is a comparatively insoluble substance — insoluble in water and dilute acids, soluble in dilute alkaline fluids; also that its reduction to the metallic state quickly follows ingestion and that its absorption is therefore comparatively slow and very incomplete. The odor of methyl telluride in the breath proved that some tellurium had been absorbed, but much of the tellurium was eliminated in the fæces in metallic form, a fact which will be referred to again.

The slightly increased elimination of nitrogen during the second period cannot be attributed solely to the influence of tellurium, because of the lack of food on the second day, and the excessive amount of food on the third day of that period. It is very well known that unusual amounts of ingested proteid stimulate nitrogenous catabolism and cause immediate increase in the output of urea; also, that when no food is eaten proteid catabolism, although diminished, still continues. In this experiment we could not well avoid a combination of both circumstances. The animal had been brought into nitrogenous equilibrium. On the second day of the tellurium period, however, when no food was retained, proteid catabolism continued at the expense of the body proteid. On the third day much of this lost proteid was made up from that ingested, but undoubtedly a good proportion of the nitrogen of the double quantity of food on this day was quickly passed into the urine. Nitrogenous equilibrium was probably very soon restored, but the small balance of 3 gms. in favor of excreted nitrogen was doubtless largely due to enforced irregularity in the feeding on the second and third days of the period.

The fæces, also, it will be seen, were not greatly altered chemically, although they were considerably increased in quantity. The percentage of nitrogen rose somewhat during the second period, but this increase was probably due to the greater quantity of mucus eliminated, to which we have already drawn attention, and was not a result of impaired digestion. There seems to have been a slight interference with the absorption of fat, since the quantity of

ether-soluble matter is somewhat increased in the second and third periods. This fact seems to harmonize with the cause assumed for increase in the fæcal nitrogen, for since tellurium is deposited in the mucous membrane of the stomach and intestines, and thereby increases the number of cells and the quantity of mucus thrown into the canal, it can be safely argued, that it may in some measure interfere with absorption. However, this increase in the quantity of ether-soluble matter, in the fæces, like the increase of nitrogen, is so slight that little importance can be attached to it.

We have already called attention to the bluish-black appearance of the fæces after administration of tellurous oxide. The color is due to metallic tellurium present in fairly large proportion. Since only traces of tellurium were present in the urine early in the oxide period, and none could be separated from a little more than 10 gms.

Periods.	Grams.			Per cent.	
	Fæces.	Ether-sol. matter.	Nitrogen.	Ether-sol. matter.	Nitrogen.
Fore	46.67	12.626	2.374	27.1	5.1
Tell. oxide	81.24	27.308	5.154	33.6	6.3
After	64.33	20.110	3.291	30.1	5.1

of hair shed during the same time, it seems very probable that the comparatively small quantity of tellurium which succeeded in getting through the walls of the intestine was finally converted into methyl telluride and that it was all being gradually eliminated in that form through the lungs. The largest proportion left the body in the fæces.[1]

Second Experiment; with Tellurous Oxide.

Although the analytic results of the first experiment indicated that there had been but slight stimulation of catabolism, we felt it desirable to make a second trial with tellurous oxide. In this second experiment we sought to avoid the vomiting which in the first had temporarily upset the equilibrium, while at the same time we aimed to keep the dose as large as possible in order to determine the maximum influences. We used a dog weighing approximately 10.5 kilos. Equilibrium was established in eight days. The diet consisted of

[1] See analytic results, Exp. 1, page 135.

175 gms. of prepared meat (6.121 gms. N), 40 gms. of cracker dust (0.604 gm. N), 30 gms. of lard, and 450 c.c. of water; it contained a total of 6.725 gms. of nitrogen. The experiment lasted three weeks and was divided into three periods of equal length. Throughout the second week tellurous oxide was given as before, in two equal doses averaging 0.21 gm. per diem; and each day there was retained 0.05 gm. more than in the previous experiment. The largest single dose was 0.15 gm., the smallest 0.05 gm.

On the fifth day, when a total of 0.3 gm. was given, the dog ate with reluctance and it was only after considerable coaxing and petting that all was swallowed. Loss of appetite had also been shown, during the previous day, when an equal amount of the oxide had been administered. We assumed, therefore, that increased dosage on the following day would cause vomiting, so the daily quantity given with the food was reduced. It was evident, however, that for that particular time we had administered the maximum quantity that could be borne without toxic manifestation. Loss of appetite was evident to the end of the period in spite of reduced dosage, but appetite quickly returned when the oxide was discontinued. Within an hour after the first dose had been swallowed the garlic odor of the breath, noticed in the previous experiment, was again recognized. It remained in evidence throughout the experiment and for some days thereafter. The languor and sleepiness prominent in the first experiment were not especially noticeable in this. There was no sickness; loss of appetite was the only approach to it.

The urine was not quite as dark in color as before. Albumin, bile pigment, sugar, and abnormal sediment were absent from the urine in all cases. None of the samples of urine gave off sufficient methyl telluride to be detected by the sense of smell. The fæces were little altered, although they acquired the characteristic bluish-black appearance during the oxide period, due, as previously stated, to metallic tellurium. They contained no unusual quantity of mucus; only once was the garlic odor perceived. In this experiment also, the cast-off hair had the usual garlic odor, but we were unable to detect any appreciable quantity of tellurium in the hair shed during the oxide period.

The tables given herewith (pages 118 and 119) present the data of this experiment.[1] They show conclusively, we think, that tellurous

[1] Indoxyl was determined with uniform quantities of urine and reagents so as to make colorimetric observations directly comparable. The dry weight of the

SECOND EXPERIMENT.

Date, 1899. Feb.	Body weight. K.	Food. Nitrogen. Grams.	TeO_2. Grams.	Urine. Vol. c.c.	Sp. gr.	Reaction. litmus.	Indoxyl. Coloration.	Nitrogen. Grams.	Total P_2O_5. Grams.	Total SO_3 Grams.	Fæces. Dry weight. Grams.	Fæces. Nitrogen. Grams.
Fore Period.												
1	10.6	6.725	..	580	1016	Acid	Strong	7.002	1.283	0.691		
2	10.6	6.725	..	610	1014	Acid	Strong	7.224	1.341	0.732	11.28	
3	10.5	6.725	..	631	1015	Acid	Weak	7.138	1.076	0.704		
4	10.5	6.725	.	570	1013	Acid	Strong	6.007	0.984	0.477		
5	10.6	6.725	..	450	1016	Alkaline	Strong	5.138	0.863	0.502		
6	10.5	6.725	..	586	1015·	Alkaline	Strong	7.021	1.071	0.684	15.71	1.539
7	10.6	6.725	..	498	1014	Acid	Weak	5.378	0.793	0.472		
Tellurous Oxide Period.												
8	10.6	6.725	0.1	564	1014	Acid	Weak	5.982	0.993	0.548		
9	10.5	6.725	0.2	599	1013	Acid	Weak	7.081	1.227	0.682		
10	10.4	6.725	0.3	642	1012	Acid	Strong	7.236	1.364	0.754	14.81	
11	10.4	6.725	0.3	598	1013	Acid	Strong	7.024	1.197	0.707		

12	10.5	6.725	0.3	473	1015	Acid	Weak	5.121	0.762	0.445		
13	10.6	6.725	0.2	584	1014	Alkaline	Strong	6.434	0.889	0.578		
14	10.6	6.725	0.1	557	1016	Alkaline	Strong	6.339	0.946	0.629	10.63	1.647
After Period.												
15	10.5	6.725	..	618	1014	Acid	Strong	7.034	1.313	0.664		
16	10.5	6.725	..	550	1016	Alkaline	Weak	6.228	0.974	0.574		
17	10.4	6.725	..	637	1013	Acid	Strong	7.331	1.287	0.761	13.94	
18	10.6	6.725	..	482	1015	Acid	Strong	5.367	0.705	0.438		
19	10.5	6.725	..	598	1014	Acid	Weak	7.097	1.304	0.681		
20	10.4	6.725	..	590	1013	Alkaline	Strong	7.146	1.106	0.637	14.67	1.832
21	10.5	6.725	..	469	1015	Alkaline	Strong	5.234	0.797	0.481		
Daily averages for each of the Three Periods.												
Fore		6.725	..	561				6.415	1.059	0.609	3.85	0.220
Tellurium		6.725	0.21	574				6.460	1.054	0.620	3.64	0.235
After		6.725	..	563				6.491	1.069	0.605	4.09	0.262

oxide in quantities as large as could well be retained had little metabolic influence that could be measured chemically. Body weight was constant; volume, reaction, and specific gravity of the urine showed little variation, total phosphoric and sulphuric acids were unchanged in quantitative elimination; and nitrogenous excretion was only slightly in excess of ingestion in each of the three periods. The distribution of nitrogen in the excreta is stated in the following summary:

Total nitrogen.	Fore period. Grams.	Tellurous oxide period. Grams.	After period. Grams.
Nitrogen of food	47.075	47.075	47.075
Nitrogen of urine	44.908	45.217	45.437
Nitrogen of fæces	1.539 } 47.341	1.647 } 47.972	1.832 } 48.215
Nitrogen of hair	0.894	1.108	0.946
Nitrogen balance	− 0.266	− 0.897	− 1.140
Ratio to nitrogen ingested.	**Per cent.**	**Per cent.**	**Per cent.**
Nitrogen of urine	95.4	96.1	96.5
Nitrogen of fæces	3.3	3.5	3.9
Nitrogen of hair	1.9	2.4	2.0
Nitrogen balance	−0.6	− 2.0	− 2.4

These results are in accord with those of the previous experiment. The unimportant excess of excreted nitrogen in each period can hardly be given much significance from any standpoint, as each amount is within the ordinary limits of error in work of this kind.

It is worthy of note that no particular influence on normal putrefactive changes in the intestine was manifested, for indoxyl could be detected in every day's urine. The normal fluctuations were quite noticeable. The indoxyl reactions were obtained most distinctly on or

fæces is recorded on the day of elimination. The nitrogen of the fæces was determined in the combined excreta of each period. The average daily weight of shed hair was: fore period, 1.02 gm. (0.128 gm. N); tellurous oxide period, 1.30 gm. (0.158 gm. N); after period, 1.10 gm. (0.135 N).

about the days of defecation, indications that the formation of indigo bodies was greatest when the matter in the intestines was largest in amount. The fæces collected throughout this experiment showed even less variability than was noticed in the previous experiment. Not only were the quantities eliminated in each period approximately equal, but nitrogen content, also, was practically the same. It may be assumed, therefore, that there was little interference with absorption in this experiment.

This animal seemed to bear the tellurium dosage especially well. At the end of the equilibrium experiment 0.75 gm. of the oxide was given with the usual morning meal. It did not cause vomiting, although a few hours thereafter the odor of methyl telluride in the expired air was almost unbearable, and it remained strong for several weeks. Even languor and sleepiness were not particularly noticeable.

Third Experiment; with Sodium Tellurite and Tellurium Tartrate.

In several preliminary experiments both the tellurite of sodium and the tartrate of tellurium seemed to be more distinctly toxic than tellurous oxide, facts which are doubtless dependent on the greater solubility of the former compounds.[1] The dog weighed 9.8 kilos. Equilibrium was established in four days. The daily food was composed of 160 gms. prepared meat (5.856 gms. N), 40 gms. cracker dust (0.604 gm. N), 30 gms. lard, and 400 c.c. water. The total nitrogen was 6.460 gms. The experiment was carried through four periods, each a week in length. Throughout the second period sodium tellurite was given in meat capsules with the food as before; in the fourth, tellurium tartrate. The third or intermediate period gave the animal time to recover from any influence of the tellurite, and, serving as an "after" as well as a "fore" period, enabled us to note any possible cumulative effect of the dosage.

The largest dose of the tellurite was 0.15 gm. with half the daily quota of food, the smallest 0.05 gm. The greatest amount of tellurium tartrate given with any one meal was 0.025 gm., the smallest 0.0125 gm. On the evening of the sixth day of the sodium tellurite period, the dog ate the usual portion of food only after much persuasion. Loss of appetite was very marked. On the next day,

[1] It should not be forgotten, however, that tellurites are transformed into the hydrated oxide by the acid of the gastric juice. The oxide likewise becomes tellurite in the alkaline liquids of the intestines.

assuming that the limit of dosage had been reached, and wishing to prevent vomiting, the dose was decreased to the smallest quantity of the period. No trouble was experienced with the tellurium tartrate. We were, however, afraid to increase the dose over 0.05 gm., as 0.1 gm. had caused vomiting in another dog. Possibly for this one the dose might have been raised somewhat.

Within half an hour after the ingestion of the first dose of tellurite, the garlic odor of the breath was very noticeable. It continued throughout the whole experiment. On the day the tellurium tartrate was first administered, nothing resulted save an unmistakable increase in the odor. With the exception of the loss of appetite on the sixth day of the tellurite period, and the garlic odor of the breath, there was nothing at any time to indicate that the dog was not normal. The urine showed little variation in color and nothing abnormal could be detected in it. Even the fæces were only a little blackened by metallic tellurium; in all other outward appearances they were perfectly normal. No methyl telluride could be detected at any period in the solid excreta even directly after passage.

The accompanying tables, pages 124 and 125, giving detailed analytic data[1] for this experiment, point to the same general conclusions that were drawn from the first and second experiments. These non-toxic doses induced very little alteration in the course of metabolic events. The weight of the animal fluctuated very little; the volume, specific gravity, and reaction of the urine were practically constant throughout; and the quantity of sulphuric acid excreted was the same in each period. The nitrogen showed little deviation from the normal, although slight stimulation, after dosage, was again indicated. On page 123 are the figures for the distribution of nitrogen in the various excreta, which emphasize the conclusions already drawn.

In this experiment we determined quantitatively the amount of combined sulphuric acid in order to measure more definitely than was the case in the previous experiment the effect of tellurium on intestinal putrefaction. It will be noticed that the normal fluctuations

[1] Nitrogen was determined, every two or three days, in combined urines. (See note, bottom of page 111). Total SO_3 of the urine, and the nitrogen and ether-soluble matter of the fæces, were determined in the excreta for the whole period. Combined SO_3 was determined in the urine passed on the days of elimination and also in the combined urines of each period. Dry weight of fæces is recorded on days of defecation. The average daily amount of cast-off hair varied between 0.77 gm. and 0.89 gm.; the content of nitrogen between 0.099 gm. and 0.115 gm. The dosage appeared to have no influence in this connection.

are here again emphasized and that the combined sulphuric acid was greatest in quantity in the urine on or about the days of defecation. In only one case was the amount of combined sulphuric acid in the urine of the day of defecation less than the average daily output of

Total Nitrogen.	Periods.			
	Normal. Grams.	Sod. tellurite. Grams.	Intermediate. Grams.	Tell. tartrate. Grams.
Nitrogen of food	45.220	45.220	45.220	45.220
Nitrogen of urine	41.878	41.452	40.432	41.300
Nitrogen of fæces	3.126 } 45.757	3.896 } 46.042	3.781 } 44.934	3.812 } 45.916
Nitrogen of hair	0.753	0.694	0.721	0.804
Nitrogen balance	− 0.537	− 0.822	+ 0.286	− 0.696
Ratio to nitrogen ingested.	Per cent.	Per cent.	Per cent.	Per cent.
Nitrogen of urine	92.6	91.7	89.4	91.3
Nitrogen of fæces	6.9	8.6	8.4	8.4
Nitrogen of hair	1.7	1.5	1.6	1.8
Nitrogen balance	− 1.2	− 1.8	+ 0.6	− 1.5

the same for the whole period. The ratios of combined to total sulphuric acid are here summarized; from these it is evident that tellurium, in the quantities and forms administered, had no material influence on intestinal putrefaction.

Periods.	Grams.		Ratio.	Per cent of Total.
	Combined SO_3.	Total SO_3.	Combined to Total.	Combined SO_3.
Normal	0.361	4.461	1 : 12.4	8.1
Sodium tellurite	0.411	4.398	1 : 10.7	9.3
Intermediate	0.461	4.537	1 : 9.8	10.1
Tellurium tartrate	0.427	4.316	1 : 10.1	9.9

THIRD EXPERIMENT.

Date. 1899. April.	Body weight. K.	Food. Nitrogen. Grams.	Urine. Vol. c.c.	Urine. Sp. gr.	Urine. Nitrogen. Grams.	Urine. Total SO_3. Grams.	Urine. Comb. SO_3. Grams.	Fæces. Dry weight. Grams.	Fæces. Nitrogen. Grams.	Fæces. Ether-soluble matter. Grams.	Fæces. Ether-soluble matter. %	
Normal Period.												
18	9.8	6.460	461	1018			(0.054)	10.80				
19	9.8	6.460	360	1016	11.476							
20	9.7	6.460	503	1018								
21	9.7	6.460	430	1014	12.821		(0.059)	22.63				
22	9.7	6.460	435	1014								
23	9.8	6.460	392	1019								
24	9.7	6.460	510	1015	17.581	4.461	(0.061)	21.72	3.126	16.201	29.4	
Sodium Tellurite Period.												Na_2TeO_3. Gram.
25	9.7	6.460	450	1017								0.3
26	9.8	6.460	373	1019	11.732		(0.068)	13.94				0.1
27	9.6	6.460	535	1019								0.1
28	9.6	6.460	304	1019	11.981		(0.052)	24.62				0.1
29	9.7	6.460	440	1019								0.2
30	9.7	6.460	485	1019								0.3
May 1	9.6	6.460	446	1015	17.739	4.398	(0.084)	25.11	3.896	22.581	35.4	0.1

Intermediate Period.												
2	9.7	6.460	451	1015								
3	9.8	6.460	370	1019	11.531							
4	9.8	6.460	445	1018			(0.098)	34.61				
5	9.9	6.460	353	1016	11.764							
6	9.8	6.460	480	1016								
7	9.7	6.460	467	1016			(0.083)	25.21	3.781	19.878	33.2	
8	9.8	6.460	430	1016	17.137	4.537						
Tellurium Tartrate Period.												$Te(C_4H_5O_6)_4$. Gram.
9	9.9	6.460	415	1015								0.050
10	9.8	6.460	502	1017	12.003							0.050
11	9.7	6.460	410	1019			(0.089)	22.18				0.025
12	9.6	6.460	485	1016	12.178							0.025
13	9.6	6.460	406	1018			(0.071)	15.74				0.050
14	9.7	6.460	471	1017								0.025
15	9.7	6.460	448	1019	17.119	4.316	(0.092)	24.41	3.812	18.792	30.1	0.025
Daily averages for each of the Four Periods.												
Normal.		6.460	441		5.982	0.637	0.052	7.88	0.447	2.314	29.4	Na_2TeO_3
Sod. Tellurite.		6.460	433		5.922	0.628	0.059	9.10	0.557	3 226	35.4	0.171
Intermediate.		6.460	428		5.776	0.648	0.066	8.55	0.540	2.840	33.2	$Te(C_4H_5O_6)_4$
Tell. Tartrate.		6 460	448		5.900	0.617	0.061	8.90	0.545	2.685	30.1	0.036

The increased quantity of ether-soluble matter in the fæces, recorded in the table of the first experiment, is repeated in this experiment after the administration of the tellurium compounds. The ratio of the fat and nitrogen to the whole quantity of the fæces for each period is shown in the summary:

Periods.	Grams.			Per cent.	
	Fæces.	Ether-sol. matter.	Nitrogen.	Ether-sol. matter.	Nitrogen.
Normal	55.15	16.201	3.126	29.4	5.7
Sod. tellurite	63.67	22.581	3.896	35.4	6.3
Intermediate	59.82	19.878	3.781	33.2	6.3
Tell. tartrate	62.33	18.792	3.812	30.1	6.1

There is seen to be a rise in the quantity of both ether-soluble and nitrogenous matter during the dosage periods; this, though very slight, indicates some interference with absorption, and probably an increase in the quantity of mucus and epithelial cells. The action here may be relatively more marked because the soluble substances would naturally have more decided local action than the insoluble oxide. However, these differences are entirely too slight for more than reasonable guesses.

At the close of the experiment 0.1 gm. of tellurium tartrate given with the usual morning meal caused vomiting in little less than an hour. Two days thereafter 0.5 gm. of the tellurite produced the same effect in three hours. The odor of methyl telluride in the breath was especially strong at the time of vomiting.

Fourth Experiment; with Sodium Tellurate.[1]

With the results of the first three experiments before us it appeared altogether unlikely that non-toxic amounts of tellurates would have a more decided action than that already observed. It

[1] The preparation of tellurates in a pure condition is a most difficult problem. After working several months, with the assistance of the late Dr. Herman A. Loos, Professor Lenher succeeded in making for us 0.5 gms. of almost chemically pure sodium compound. This preparation was recrystallized at least twenty times. Its only impurity was a very small proportion of sodium tellurite. It is probable that commercial tellurates are no purer than this preparation and that their effects, when given as drugs, are modified by the small quantities of tellurite which they contain.

seemed desirable, however, to determine experimentally the influence of sodium tellurate on metabolism, because of the therapeutic employment of this particular compound. The dog used in this concluding experiment weighed 15.5 kilos.[1] The diet consisted of 275 gms. of prepared meat (9.675 gms. N), 50 gms. of cracker dust (0.755 gm. N), 30 gms. of lard and 600 c.c. of water; it contained in all 10.430 gms. of nitrogen. This diet was given for eight days, until the weight of the animal remained constant, when the experiment was begun. It was carried through three periods; the first and third were each a week in length; the second, eight days. During the second the tellurate was given daily with the food in the accustomed way. The largest dose of the tellurate, 1 gm., was given on the last day of the second period with the morning meal. With the first food of the tellurate period 0.5 gm. was given, and none for the rest of the day. The amount regularly administered was 0.25 gm. with each portion of food.

During the night of the third day the dog vomited a little greenish mucus. As this indicated cumulative action no tellurate was given on the fourth day. The vomited mucus was mixed with the food given the next morning. There were no manifestations of illness other than vomiting, and no toxic symptoms were exhibited even after the administration of the unusual dose during the morning of the last day of the tellurate period. Sleepiness, however, was very marked at the end of the second and at the beginning of the third periods. Within a very short time after the first ingestion of tellurate the alliaceous odor of the breath was very marked. It seemed to increase steadily, and was, of course, strongest after the administration of the largest dose; for more than two months thereafter it was still very perceptible.

The urine manifested the customary coloration changes — became more coffee colored with tellurium dosage — but no abnormal constituents could be detected in it, except occasionally a garlic odor.[2] Its reaction was acid throughout and indoxyl could be

[1] Six months previous to this experiment a gastric fistula had been made in this dog for experimentation in other connection. At this time the cannula had not been opened for a little more than a month. The fistula was kept closed throughout each of the three periods. The dog remained in perfectly healthy condition to the end of the experiment.

[2] By an unfortunate oversight we failed to look for tellurium in the urine. After the largest dosage it is probable that the urine did contain the substance. See results in this connection on pages 111 and 135.

FOURTH EXPERIMENT.

Date. 1900. April.	Body weight. K.	Food. Nitrogen. Grams.	Urine. Vol. c.c.	Sp. gr.	Reaction. litmus.	Total solids. Grams.	Total solids. %	Nitrogen. Grams.	Urea. Grams.	Urinary Total Solids. Nitrogen. %	Urinary Total Solids. Urea. %	Fæces. Dry weight. Grams.	Na_2TeO_4. Grams.
Fore Period.													
25	15.5	10.43	485	1025	Acid	28.27	5.83	9.61	20.45	33.99	72.34		
26	15.6	10.43	345	1028	Acid	22.50	6.52	6.69	14.24	29.72	63.29		
27	15.5	10.43	720	1021	Acid	35.23	4.89	10.20	21.71	28.96	61.62	26.7	
28	15.5	10.43	455	1028	Acid	29.68	6.52	9.08	19.31	30.58	65.06		
29	15.6	10.43	635	1021	Acid	31.07	4.89	8.91	18.96	28.68	61.02		
30	15.5	10.43	330	1036	Acid	27.68	8.39	9.86	20.97	35.61	75.76		
May. 1	15.6	10.43	405	1025	Acid	23.59	5.83	9.15	19.46	34.78	74.00	32.7	
Sodium Tellurate Period.													
2	15.7	10.43	395	1026	Acid	23.93	6.05	8.62	18.33	36.00	76.60		0.5
3	15.6	10.43	610	1024	Acid	34.11	5.59	11.93	25.38	34.97	74.41	18.3	0.5
4	15.5	10.43	540	1025	Acid	31.45	5.83	12.10	25.75	38.48	81.87		0.5
5	15.7	10.43	335	1032	Acid	24.98	7.46	9.40	20.00	26.87	80.07		
6	15.6	10.43	645	1024	Acid	36.07	5.59	10.99	23.40	30.49	64.88	28.1	0.5
7	15.8	10.43	270	1034	Acid	21.39	7.92	8.81	18.75	41.20	87.66		0.5
8	15.7	10.43	535	1028	Acid	34.90	6.52	12.45	26.50	35.69	75.93		0.5
9	15.6	10.43	445	1034	Acid	35.25	7.92	12.52	26.64	35.52	75.57	31.4	1.0

After Period.

10	15.7	10.43	380	1031	Acid	28.45	7.22	10.53	22.41	37.02	78.77		
11	15.5	10.43	575	1024	Alkaline	32.15	5.59	10.07	21.43	31.32	66.65	28.3	
12	15.6	10.43	460	1029	Acid	31.08	6.76	10.73	22.84	34.53	73.48		
13	15.6	10.43	550	1022	Alkaline	28.19	5.13	9.60	20.44	34.07	72.50	16.4	
14	15.7	10.43	410	1028	Acid	26.75	6.52	8.68	18.47	32.45	69.05		
15	15.6	10.43	290	1042	Acid	28.38	9.79	10.55	22.45	37.18	79.11		
16	15.5	10.43	590	1029	Acid	39.87	6.76	11.26	23.97	28.26	60.13	21.4	

General Summaries.

Periods. I. Totals.	Nitrogen. a. Food. Grams	Nitrogen. b. Urine. Grams.	Nitrogen. Ratio. a : b.	Nitrogen. Difference. Grams.	Urine. Vol. c.c.	Urine. Total solids. Grams.	Urine. Total solids. %	Urine. Urea. Grams.	Urine. Urea. % of T. S.	Fæces. Dry weight. Grams.	Na_2TeO_4. Grams.
Fore, 7 days	73.01	63.50	1 : 0.87	+ 9.51	3375	198.02	5.87	135.10	68.2	59.4	
Tellurate, 8 days	83.44	86.82	1 : 1.04	— 3.38	3775	242.08	6.41	184.75	76.3	77.8	4.0
After, 7 days	73.01	71.42	1 : 0.98	+ 1.59	3255	214.87	6.60	152.10	70.7	66.1	
II. Averages. Fore	10.43	9.07	1 : 0.87	+ 1.36	482	28.29	5.87	19.30	68.2	8.5	
Tellurate	10.43	10.85	1 : 1.04	— 0.42	472	30.26	6.41	23.09	76.3	9.7	0.5
After	10.43	10.20	1 : 0.98	+ 0.23	465	30.70	6.60	21.71	66.1	9.4	

detected in each sample. The fæces contained a little more than the normal amount of mucus, during the second and part of the third period, and the bluish-black color of deposited tellurium which had been noticed before was again observed; otherwise there was nothing unusual to be noted.

In this experiment nitrogenous metabolism was measured by the output of nitrogen in the urine only. The nitrogen was determined by the hypobromite method. Urea was calculated from the nitrogen. The accompanying tables summarize the data of this experiment (pages 128 and 129).

Here again the results are essentially a repetition. Body weight as well as the volume, reaction and specific gravity of the urine were unaffected. The total solids of the daily urine were practically the same in each period, but nitrogen (urea) was increased enough to indicate, as in the case of all of our previous experiments, that metabolism had been slightly stimulated. With the exception of the vomiting on the third day and the continuous elimination of methyl telluride in the expired air, there were no visible toxic effects of the tellurate. The dog was particularly sleepy for a short time, as already mentioned, but did not suffer from loss of appetite, a symptom observed in each of the preceding experiments. In fact, the tellurate seemed to be especially devoid of toxicity, for even 1.5 gm. given on an empty stomach with a small piece of meat at the close of the experiment, caused vomiting only after seven hours. The quantitative eliminations of the fæces, it will be seen from the tables, were so constant that it may safely be said that no particular effect was produced on intestinal absorption, except, perhaps, a slightly diminished assimilation of fat.

Review.

In reviewing the results of these metabolism experiments it should be mentioned that the occasional vomiting was quite in accord with the original observations of Hansen (2) and the experience of subsequent workers. The alliaceous odor of the breath after the introduction of tellurium has been observed by all investigators except Rabuteau (3) and Combemale and Dubiquet. Reisert (4), inquiring into the cause of the so-called bismuth breath, found that when men took only 0.000,000,5 gm. of tellurous oxide, in solution, the odor of garlic could be noticed in the breath 75 minutes after-

ward, and that it continued for about 30 hours. Before Wöhler and Dean's[1] and Heeren's[2] observations were made this odor had been attributed to ethyl telluride by Wöhler and his pupils.[3] Heeren assumed that the volatile substance exhaled was in reality the telluride of methyl. Hofmeister (12) has lately proved by chemical means that synthesis of methyl telluride occurs in almost all parts of the body after the introduction of tellurium in any form, and Beyer has found that the process does not take place in the absence of oxygen. Hofmeister has also shown that methyl telluride is formed in worms and crustacea, as well as in dogs and rabbits, and Hofmeister and Czapek and Weil observed similar production after administration of tellurium to frogs. Neither Knop (5) nor Bokorny (11), who have found that small quantities of tellurium compounds exert little or no destructive action on plants, observed this synthesis on the part of vegetable cells.

The very evident languor, sleepiness, and loss of appetite in some of these experiments, first noted by Gmelin (1), were reported by Hansen among the results of experiments on himself, and were observed also by Neusser. The color and odor of the urine and fæces, the increase of mucus, and the presence of tellurium metal, in the latter, confirm previous observations by Hansen, Beyer and Reisert. The latter found that the garlic odor, after ingestion of 0.015 gm. of tellurous oxide, could be perceived in the urine 382 hours; in the sweat, 452 hours; in the fæces, 79 days. In the breath it was still present at the end of 237 days.[4]

Tellurium appears to have had no influence at all on intestinal putrefaction. This result, however, harmonizes with the very recent observations of Scheurlen (14) and Klett (15), who found that the development of various forms of bacteria, for example, Staphylococcus

[1] Wöhler und Dean: Annalen der Chemie und Pharmacie, 1855, xciii, p. 233.

[2] Heeren: Chemisches Centralblatt, n. F., 1861, vi, p. 916.

[3] Wöhler: Annalen der Chemie und Pharmacie, 1840, xxxv, p. 111; *Ibid.*, 1852, lxxxiv, p. 69. Also, Mallet: *Ibid.*, 1851, lxxix, p. 223. Also, Wöhler: Journal für praktische Chemie, 1840, xx, p. 371.

[4] We are greatly indebted to Professor John Marshall for calling our attention to Reisert's work. It seems that subsequent foreign investigators of the behavior of tellurium in the animal body were unaware of Reisert's results. It is probable, however, that Kunkel refers to these results when he says, "The odor (of methyl telluride) has been detected in the fæces of man over two months and in the breath more than a half-year, after the last dose of tellurium." Handbuch der Toxicologie, erste Hälfte, 1899, p. 365.

pyogenes aureus and B. mesentericus vulgatus, was not materially hindered by small proportions of tellurite. Klett observed that the virulence of such bacteria as B. anthracis was not perceptibly decreased by the action of small quantities of the same salt.[1] In all of our experiments much of the ingested tellurium was quickly transformed to the passive metallic state. As a consequence, the proportion of active tellurium in the intestinal contents must have been very slight.

Attention has already been called to the fact that Beyer's brief and imperfect experiment on the excretion of urea after intravenous injection of sodium tellurate was the only previous attempt to deter-

Tellurium compound used.	Nitrogen ingested daily. Grams.			Nitrogen excreted daily. Grams.			Total balance of nitrogen for each period. Grams.		
	Fore.	Dosage.	After.	Fore.	Dosage.	After.	Fore.	Dosage.	After.
1. TeO_2	9.854	10.839	9.854	9.827	11.147	9.667	+0.191	−3.079	+1.309
2. TeO_2	6.725	6.725	6.725	6.763	6.853	6.888	−0.266	−0.897	−1.140
3. Na_2TeO_3	6.460	6.460	6.460	6.537	6.577	6.419	−0.537	−0.822	+0.286
$Te(C_4H_5O_6)_4$	6.460	6.460	..	6.419	6.559	..	+0.286	−0.696	
4. Na_2TeO_4	10.430	10.430	10.430	9.070	10.850	10.200	+9.510	−3.380	+1.590

The figures for excreted nitrogen in Experiment 4 represent only that eliminated in the urine, so that the corresponding figures under "total balance" represent differences between food and *urine* nitrogen.

mine the metabolic influence of tellurium. He found that the normal amount of urea eliminated in the urine of a healthy dog during three preliminary days was 9.45, 10.41 and 7.62 per cent respectively, an average of 9.16 per cent. After injection of 0.75 gm. of sodium tellurate (0.27 gm. tellurium) into the jugular vein the urea in the urine on five successive days was 1.79, 6.06, 8.50, 7.98, 9.00 per cent, an average of 6.67 per cent. This falling-off in the amount of urea was due, undoubtedly, to the refusal of the dog to eat on the first and second days of the tellurium period, and as Beyer does not give any analytic data regarding the food, it is impossible to attach any

[1] Our attention was first called to the work of Scheurlen and Klett by Dr. P. H. Hiss, to whom we are also indebted for valued suggestions.

special importance to his results in this connection. After the injection of tellurium, albumin and bile pigment were eliminated in the urine for several days. On the first day after injection of tellurate, 0.062 gm. of metallic tellurium was eliminated, on the second 0.081 gm., on the third a trace. More than half the amount injected, therefore, was eliminated through the kidneys.

Our own results with respect to nitrogenous catabolism are shown in the above general summary, page 132.

II. Influence on Digestion and on the Gastro-Intestinal Tract in General.

In our metabolism experiments we noted that vomiting occurred in the first and fourth experiments, soon after ingestion of 0.5 gm. of tellurous oxide and several hours after 0.25 gm. of sodium tellurate had been administered. At times there was loss of appetite and in practically all of the experiments the elimination of mucus in the fæces was increased. We saw, also, that tellurium compounds were reduced in the gastro-intestinal tract, that absorption of fat was diminished and that methyl telluride mingled with the fæcal gases. We have attempted to determine by additional experiments some of the other special influences of compounds of tellurium in the alimentary tract.

Experiments on the Normal Dog.[1]

The following abbreviated reviews present the essential points observed in this connection, together with other facts of interest:

1. **With tellurous oxide.** *1899. Jan. 7.* — Dog weighed 14 kilos. Had received no food during previous 24 hours. Was given a total of 3.5 gms. of TeO_2, with 280 gms. fresh meat, in equal portions — 0.5 gm. TeO_2 in pieces of meat weighing 40 gms. — at 1.30, 3.30, 5.00, 8.15, 9.15, 10.15, and 10.45 P. M. Drank 200 c.c. water with first dose. Odor of methyl telluride in room very strong at 2.30 P. M. At 9.00 animal very sleepy and odor sickening. Continued so throughout experiment. At midnight had neither vomited nor passed urine. *Jan. 8.* — Considerable vomit found in morning; full of undigested pieces of meat, with heavy white and greenish black mucus. Contained much undissolved TeO_2. Was acid to litmus; no free acid. Dog very languid.

[1] The dogs of these experiments were kept in the cage used in the metabolism work. Its arrangement favored separation of solid matter in the vomit from fluid, as well as the separation of fæces from urine. Tests for free acid were made with Günzburg's reagent and tropæolin 00.

12.30 P. M., first food offered — 20 gms. meat with 0.5 gm. TeO_2 — eagerly eaten; water refused. 1.45, all vomited, with much greenish black mucus in strings and lumps. Acid to litmus; no free acid. Contained undissolved TeO_2. 11.30 P. M. (no food or water during interval), vomited again. Mostly clear fluid with much mucus. Acid to litmus; no free acid. *Jan. 9.* — 10 A. M., drank 500 c.c. water; no food given. Ten minutes later 100 c.c. vomited: neutral to litmus. 10.30, 175 c.c. urine eliminated. The urine yellowish green, like diluted bile, though no bile pigments were present. No coagulable proteid. 150 gms. meat at 6 P. M. *Jan. 10.* — 150 gms. meat, 200 c.c. water at 9 A. M. 30 gms. meat with 0.5 gm. TeO_2 at 5.15 P. M. At 8.00, fæces — bluish-black, streaks of blood, much mucus. Urine also, 80 c.c., not as dark in color as on 9th. No coagulable proteid. 8.30, 30 gms. meat with 0.5 gm. TeO_2. *Jan. 11.* — No vomiting since last doses. 11 A. M., refused food and water — none for 26 hrs. Nose very warm and dry. Refused food repeatedly all day. Persisted in sleeping. Fever high at midnight. Dog not easily roused from stupor. *Jan. 12.* — 9 A. M., 100 gms. meat, in several pieces, eaten; vomited in 10 minutes. Solid portion eaten; again quickly thrown up. This occurred three times in half hour. Fluid each time acid to litmus; no free acid. Greenish mucus very abundant. 2 P. M., vomited again; acid to litmus; no free acid. *Jan. 13.* — Ate small quantities of meat and drank water, with increasing appetite throughout day. 300 c.c. urine in morning; not particularly dark. *Jan. 14.* — Recovering rapidly. Odor of methyl telluride undiminished. *Jan. 15.* — 10 A. M., 50 gms. meat in one piece with 1.0 gm. TeO_2 and 200 c.c. H_2O. 12 M., 60 gms. piece with 1.0 gm. TeO_2. 4 P. M., 30 gms. piece with 1.0 gm. TeO_2. Up to midnight no action except increased methyl telluride and stupor. *Jan. 16.* — Vomit found at 9 A. M. — 30 and 40 gms. pieces meat unchanged, with contained TeO_2 in place. Greenish fluid, full of greenish and bluish shreds of mucus. Strongly acid to litmus; no free acid. Urine normal in appearance, 250 c.c. at 9.30. At 12.30 P. M., vomited again. 60 gms. piece meat thrown up, undiminished in size; putrid. Strings of blue mucus half foot in length. Some TeO_2 undissolved. Vomit acid to litmus, none free. 5.00, bloody fæces; bluish-black in places. *Jan. 17.* — 9 A. M., unusually lively. 30 gms. meat, 1.0 gm. TeO_2, 200 c.c. water. 3 P. M., fluid vomit; green and blue mucus; acid to litmus, none free. 5.00, tried to vomit, without success. 5.30, vomited 30 gms. piece meat given at 9 A. M. TeO_2 powder in blue mucus. Acid to litmus; none free. Midnight, 115 c.c. very dark urine. Contained coagulable proteid; no bile pigment.

Jan. 18. — *Post-mortem* (chloroform, 9 A. M.). Methyl telluride from abdominal cavity and separate organs. Blood, liver, lungs, brain, spleen, normal in outward appearance. Gall bladder greatly distended. Alimentary tract lined throughout with greenish and bluish-black layer of metallic tellurium in granules. Small intestines much inflamed. Contents of stomach acid to

litmus; no free acid. Pepsin present. Intestinal contents bluish-black; much mucus. Kidneys very dark, cortical layer black. Urine in bladder very dark; no tellurium in suspension. Walls of bladder normal in appearance.

Analytic results. Qualitative analysis of various parts by method outlined on page 109 gave following results for tellurium: *positive*, liver, blood, stomach, intestines, muscle from back, urine, contents of stomach and of large and small intestines, bile, fæces; *negative*, lungs, spleen, pancreas, brain, heart. The amount in the fæces was surprisingly large, 75 gms. of the desiccated material yielding 0.977 gm. of tellurium — 1.3 per cent of dry substance.[1]

2. **With tellurous oxide.** *1899. Mar. 13.* — Bitch weighed 16 kilos. 9.30 A. M., 1.0 gm. TeO_2, 125 gms. meat, 300 c.c. H_2O. Ten minutes later nearly all vomited; all solid portion licked up at once. At 11.00, large quantity thrown up again; all eaten quickly. This repeated at 3.00, 6.30, 8.45 and 11.15 P. M. Vomit less and less each time; proportion of bluish-black mucus correspondingly greater. Samples of each vomit acid to litmus; no free acid. No haemoglobin in any, but bile pigment in some. Increasing number of bacteria. Each gave good precipitate with $AgNO_3$ and HNO_3 after removal of albuminate and proteose. Kelling's and Uffelmann's [2] tests for lactic acid gave negative results. Urine had usual coffee color. Odor of telluride of methyl very strong soon after first dosage.

3. **With sodium tellurite.** *1899. Apr. 7.* — Bitch weighing 6.2 kilos. 9 A. M., full meal meat, bread, water. 3.30 and 4.30 P. M., 15 gms. meat enclosing 0.1 gm. Na_2TeO_3. 5.30 and 6.30, same quantity meat with 0.25 gm. Na_2TeO_3. At 4.00, methyl telluride very noticeable about cage; more and more intense throughout day. 6.35, vomit — fluid and mucus. Acid to litmus, no free acid. 7.35, more vomit — three pieces of meat given during afternoon thrown up little altered, with parts of fourth. Blue mucous strings. Fluid acid to litmus; none free. 9.30, refused food. Sleepiness pronounced. 10.00, 150 c.c. urine, somewhat darker, otherwise normal.

4. **With tellurium tartrate.** *1899. Apr. 8.* — Same dog used Apr. 7th, 8.30 A. M., urine normal in appearance. 9.30, 100 c.c. water, 15 gms. meat in piece enclosing 0.3 gm. $Te(C_4H_5O_6)_4$. 10.45, same quantity water and meat with 0.43 gm. $Te(C_4H_5O_6)_4$. Methyl telluride stronger an hour after first dosage. Vomit at 11.15, 11.30, 11.40, 11.55 A. M., and 12.10 and 12.35 P. M. Unchanged pieces of meat came up at 11.15 and 11.30 A. M. Much fluid and mucus thereafter. Each vomit acid to litmus, with no free acid. Dog very ill during afternoon; recovered rapidly during evening. At first refused food. 10 P. M., ate largely and eagerly; food retained. 11.30, 125 c.c. normal urine.

5. **With sodium tellurate.** *1900. Apr. 16.* — Dog weighed 7.3 kilos. Good meal night before. 12.30 P. M., 0.5 gm. Na_2TeO_4 with 100 gms. meat,

[1] See quantitative results on page 143.

[2] SIMON: A manual of clinical diagnosis, 1897, pp. 156–157.

two pieces. Methyl telluride very strong within an hour. 5 P. M., 0.5 gm. Na_2TeO_4 in 100 gms. meat, three pieces. Sleepiness very marked, odor unusually strong at 7 P. M. No other marked symptoms. *Apr. 17.* — 9 A. M., odor of methyl telluride in room almost unbearable.[1] No food given. Very sleepy. 10.00, vomited — two pieces meat each weighing nearly 30 gms., with considerable quantity grayish-black mucus. Vomit acid to litmus; none free.

Post-mortem (Chloroform, 11 A. M.). Only pathological conditions noted were inflammation of intestines; bluish-black lining of gastro-intestinal tract due to granular tellurium in epithelium; and methyl telluride from abdominal cavity and organs. No tellurium could be separated from the lungs.

Many of the results in the above experiments confirm observations made in our metabolism experiments and in those of previous investigators, especially Hansen, Rabuteau and Beyer; but particularly striking is the fact that there was never any free acid in any of the mixtures thrown from the stomach. It is quite evident from these experiments that irritation of the gastric mucous membrane is usually very marked, although it required at times a surprisingly large quantity of tellurium compound to cause irritation. The intestines were also much inflamed by tellurium. The mucous cells appeared to be greatly stimulated, judging from the large quantities of mucus secreted. Slight intestinal hemorrhage was also produced, as was occasionally shown by the bloody fæces. The results of each of these experiments seem to combine to prove that tellurium exerts an inhibitory action on the secretion of acid in the stomach. Certainly not enough acid is found to furnish free acid, even when only a small amount of proteid is present there to combine with it. This must be one of the causes of the indigestion repeatedly observed throughout these experiments. It does not seem probable that mere transformation of the small quantity of tellurium compounds administered could account for the disappearance of free acid. We have not recorded, above, the individual results regarding the presence of proteolytic enzyme. Pepsin was contained in active quantity in each particular

[1] A dog of 15 kilos weight which had been perfectly healthy during the six months he was in our charge was chained near the animal on which the experiment was being performed. During the night he vomited twice. This seemed to be due entirely to inhaled methyl telluride. The windows and doors of the room had been closed for the night, so that the telluride accumulated. See personal reference on page 147.

vomit. When an equal amount of 0.2 per cent hydrochloric acid was added, giving distinct blue reaction with congo red, fibrin in relatively large quantity was quickly digested in all samples.

Experiments on a Dog with Gastric Fistula.

In order to test the above conclusion regarding interference with secretion of hydrochloric acid, we conducted on a dog with gastric fistula some experiments designed to give even more direct evidence in this connection. The dog weighed 15.5 kilos. The cannula was put in place, toward the pyloric end, on the 9th of November, 1899, five weeks before the experiments were begun. Entire recovery speedily resulted and the dog seemed to digest normally.[1]

1. **Preliminary control experiments.** — *I. 1899. Dec. 15.* — 12.15 P.M., free acid in stomach contents. 12.45, 150 gms. of meat given in four pieces of equal size. 5.50, free acid in contents. Time from feeding to first appearance of free acid, 5 hr. 55 min.[2]

II. 1899. Dec. 17. — 9.50 A.M., free acid in stomach contents. 10.00, 50 gms. of meat given in one piece. 12.00, free acid detected. Time required for appearance of free acid, 2 hr. 0 min.

2. **Experiments with tellurium compounds.** — *I.* **With tellurous oxide.** — *1899. Dec. 18.* — 9 A. M., free acid in contents of stomach. 9.15, fed 150 gms. of meat in four pieces of equal size, each containing 0.1 gm. TeO_2. 2 P.M., some undissolved TeO_2 in contents. 3.00, vomited small amount of thick mucus. Stomach contents scanty. Was given 25 c.c. H_2O. 4.20, drank 150 c.c. H_2O. 7.00, stomach contents faintly alkaline to litmus. 9.30, still no free acid. Contents neutral to litmus. 9.30, time since ingestion of food, with no free acid, 12 hr. 15 min. At 9.30 P. M., 50 gms. of meat given in one piece with 100 c.c. water. 10.30, free acid. The fresh meat seemed to act as a special stimulant, and in the absence of the oxide, which we assume had been mostly removed, was able to call forth abundant secretion of acid.

II. **With sodium tellurite.** — *1899. Dec. 19.* — 10.30 A. M., no free acid in stomach. Given 50 gms. of meat in single piece with 0.1 gm. Na_2TeO_3. 12.45 P.M., trace of free acid. Interval to appearance of free acid, 2 hr. 15 min.

[1] *Methods.* On the day preceding each experiment the dog was well fed and received all the water it desired. On the day of the experiment only the meat mentioned in the above summaries was fed; no water was given except when specially recorded. About 10–15 c.c. of fluid were taken from the stomach at intervals of from 15 minutes to an hour. Acidity to litmus, congo red, Günzburg's reagent and tropæolin 00 was determined qualitatively in each sample withdrawn.

[2] See Chittenden, Mendel, and Jackson: This journal, 1898, i, p. 194. The time until free acid appears is here lengthened, probably because no fluid was ingested. Note, however, the result of our last control experiment.

III. **With tellurium tartrate.** — *1899. Dec. 20.* — 10 A. M., no free acid in contents. 10.15, 150 gms. of meat in four pieces, equal in size, with total of 0.3 gm. $Te(C_4H_5O_6)_4$. 10.15 P. M., still no free acid. 10.30, interval of no free acid, 12 hours. At 10.30, 50 gms. of meat given in one piece. 12.15 A. M., no free acid. Experiment discontinued. These results might indicate that tellurium tartrate has even more decided inhibitory action than the oxide.

IV. **With sodium tellurate.** — *1900. May 25.* — 10 A. M., no free acid in contents. 10.15, 50 gms. of meat in single piece with 0.3 gm. of Na_2TeO_4. 2.15 P. M., first appearance of free acid. First appearance of free acid at the end of 4 hours.

V. **With sodium tellurite.** (Direct continuation of Exp. IV.) — 2.45 P. M., abundance of free acid. 3.00, 100 gms. meat in two pieces, with 0.3 gm. Na_2TeO_3. 10.15, first trace of free acid. First trace of free acid after an interval of 7 hr. 15 min.

Note. — The odor of methyl telluride in the exhalations always became more pronounced an hour or two after the ingestion of the meat containing the tellurium compounds. Frequently bile pigment was detected, with Gmelin's test, in the stomach contents after tellurium dosage, but not at any other time. All of the various samples tested contained pepsin which, after the addition of an equal quantity of 0.2 per cent HCl, showed vigorous digestive action on fibrin shreds. Contents almost always acid to litmus.

3. **Final control experiment.** — *1900. June 1.* — 11.15 A. M., no free acid in contents. 11.30, 50 gms. of meat fed; one piece. 11.45, free acid. Same at 12.00, 12.30 and 1 P. M. Time from feeding till free acid was detected, 15 min.

No.	Meat. gms.	Time of feeding.	First trace free acid.	Time interval.	Conditions.	Average interval.
1 (II)	50	10.00 A.M.	12.00 M.	2 hr. 0 min.	Prelim. control	1 hr. 7 min.
3	50	11.30 A.M.	11.45 A.M.	0 hr. 15 min.	Final control	
1 (I)	150	12.45 P.M.	5.50 P.M.	5 hr. 55 min.	Prelim. control	5 hr. 55 min.
2 (II)	50	10.30 A.M.	12.45 P.M.	2 hr. 15 min.	0.1 gm. Na_2TeO_3	3 hr. 7 min.
2 (IV)	50	10.15 A.M.	2.15 P.M.	4 hr. 0 min.	0.3 gm. Na_2TeO_4	
2 (V)	100	2.45 P.M.	10.15 P.M.	7 hr. 15 min.	0.3 gm. Na_2TeO_3	7 hr. 15 min.
2 (I)	150	9.15 A.M.	*	12 hr. 15 min.†	0.4 gm. TeO_2	12 hr. 7 min.§
2 (III)	150	10.15 A.M.	*	12 hr. 0 min.†	0.3 gm. $Te(C_4H_5O_6)_4$	

* No free acid when experiment was discontinued. † At least. § Minimum.

Direct comparison of the results, in the preliminary and final "control" experiments with those in which the meat fed contained tellurium, clearly brings out the fact that free acid invariably appeared in shorter time when no tellurium was given. The above summary of these experiments, page 138, in which the results for equal portions of meat are grouped together, shows this, and our data indicate, we think, that the secretion of hydrochloric acid in the gastric juice is markedly inhibited by tellurium compounds.

Influence on Zymolysis.

All evidence in our experiments up to this point, bearing on digestive conditions, appeared to favor the view that tellurium compounds, in the quantities given, have no special inhibitory action on pepsin proteolysis in the presence of free hydrochloric acid. The secretion of pepsin did not seem to be materially affected. When it is recalled, however, that traces of pepsin manifest great proteolytic power under favorable conditions, it cannot be safely inferred, from any results we have presented, that its secretion was not interfered with. In the case of the acid, however, its more definite quantitative relationship to proteolysis in the stomach makes deduction regarding its formation in these experiments much more reliable.

With a view of ascertaining roughly the action of percentages of tellurium compounds, equal to and somewhat higher than those in the stomach throughout the previous experiments, we conducted a few test tube experiments with "pepsin — HCl" and fibrin, and then, incidentally, also determined the effects of similar quantities on ptyalin and trypsin under appropriate artificial conditions.[1] We give our results briefly in summary:

1. Pepsin — HCl, 0.2%.
 I. With sodium tellurite. (Alkaline in reaction to litmus. In quantities above 0.6%, is transformed in great part into hydrated TeO_2, which

[1] *Methods.* I. "Pepsin — HCl" was prepared by dissolving 0.5 gm. of pepsin scales (P. D. & Co., 1–2000) in a litre of 0.2 per cent HCl. II. Neutral solution of trypsin was made by Kühne's method. (Given in Studies from the Yale Laboratory of Physiological Chemistry, vol. i, p. 101.) III. Neutralized, filtered saliva was used in the amylolytic experiments. IV. Proteolysis was determined by the disintegration and disappearance of purified fibrin in shreds; amylolysis on starch paste, 0.5 per cent, with iodine and Fehling's solutions as indicators. The volumes of the digestive mixtures were 15–20 c.c. Time: usually 30 minutes to an hour, at 40° C. In all cases control experiments were made to determine the activity of the enzyme solutions.

is precipitated. Reaction of mixture also becomes alkaline). Digestive action quickly obtained with amounts not over 0.625%. In presence of this quantity some acid is uncombined.

II. With tellurium tartrate (acid). Rapid digestion with as much as 1.25%.[1]

III. With sodium tellurate (containing trace of tellurite; slightly alkaline). Digestion with 1.25%.

2. Trypsin (neutral).
 I. With sodium tellurite. Rapid digestion in presence of 2.50%.[1]
 II. With tellurium tartrate. Some digestion in presence of 0.85%.
 III. With sodium tellurate. Rapid digestion in presence of 2.50%.[1]
3. Ptyalin (neutral).
 I. With sodium tellurite. No digestion with quantities above 0.02%.
 II. With tellurium tartrate. No digestion with quantities above 0.02%.
 III. With sodium tellurate. No digestion with quantities above 0.35%.

It seems quite evident, from these results, that pepsin and trypsin are not destroyed by quantities of tellurium compounds under 0.6 per cent and are active with as much as 1.25 per cent and 2.5 per cent, respectively, of some compounds. Ptyalin appears to be the most sensitive to destructive influence, trypsin least so. The reactions of the compounds appear to influence greatly these results, the tellurate (only very faintly alkaline from admixed tellurite) having the least destructive action. It may be reasonably concluded, then, that interference with digestion in the dog, after dosage with comparatively small amounts, has resulted more from disordered secretion than from direct influence on zymolysis itself.

Effect on Absorption and on the Fæces.

From the experimental data here presented we can draw hardly more than very general deductions regarding influence on absorption. The chief evidence of disturbed absorptive function is given in the figures for ether-soluble matter in the fæces of the first and third metabolism experiments, indicating decreased fat assimilation. During the dosage periods the cells of the villi take up metallic tellurium and their absorbing capacity may therefore be much diminished. The variations in nitrogen content of the fæces shown in the tables of the first three metabolism experiments are too slight to warrant the conclusion that food proteid had accumulated in the intestines. Besides, it has been very evident, in almost all our experiments that the secretion of mucus was considerably increased in the presence of tellurium, and the larger quantity of nitrogen in the fæces after dosage

[1] Effects of larger quantities were not determined.

may have been due entirely to that cause. It is perhaps unwise, however, in the absence of direct experimental evidence, to lay any stress on these points, since the digestive and absorptive changes in the intestines are far too complex, and are influenced by too many interdependent relations, for us to ascribe the increase of ether-soluble matter and nitrogen of the fæces to any one general disturbance, or to consider it a result of any specific abnormality.

Since secretion of acid in the stomach is interfered with, it may be reasonably supposed that secretory inhibition results in the intestines also and that perhaps digestion of fat was retarded for that reason. Certain it is, at all events, that loss of appetite, gastric indigestion, irritant action resulting in vomiting and disturbed secretion of gastric juice, result from sufficient dosage of tellurium; that the mucous cells in the membrane lining the gastro-intestinal tract throw out an abnormal quantity of their product; that excessive doses of tellurium may cause intestinal hemorrhage; that the cells of the mucous membrane reduce tellurium compounds to the metallic state; and that the fæces, somewhat more bulky in the dosage periods, carry off, in the form of the metal, much of the ingested tellurium. Intestinal putrefaction does not seem to be especially influenced, and methyl telluride is formed somewhere in the tract and eliminated in part, at least, per rectum.

III. Effects and Distribution after Subcutaneous Injection.

No effort has previously been made to determine quantitatively the distribution of tellurium, although its presence in almost all parts of the body, after intravenous injections, has been shown quite satisfactorily by histological methods. We give here the toxicological data of one experiment in which tellurium tartrate was injected under the skin, together with the results of some analyses of the glands and tissues.

1. **Injection experiment. With tellurium tartrate.** *1899. April 9.* Bitch weighed 6.2 kilos (same animal had previously been used; in experiments 3 and 4, page 135). 10 A.M., full meal given. 3.30 P.M., 0.25 gm. $Te(C_4H_5O_6)_4$ (5 c.c. of 5 per cent sol.) injected on side, posteriorly. Marked local irritant action. 3.50, very restless. 4.00, tremor in limbs. 4.10, garlic odor very strong. 4.20, tongue and jaws moving continually, as if to get rid of ill-tasting matter. 4.30, 0.2 gm. $Te(C_4H_5O_6)_4$ injected, near same place (4 c.c. of 5 per cent sol.). 4.50, breathing more labored. 5.10, muscles

twitching all over body. 5.30, 1.0 gm. $Te(C_4H_5O_6)_4$ injected, opposite side (5 c.c. of 20 per cent sol.). 6.00, very unsteady. 6.20, movements of tongue and jaws less frequent. 8.30, stupor; aroused with difficulty. 8.45, 90 c.c. urine — coffee colored, containing coagulable proteid and bile pigment; no sugar. (Urine, night before, normal.) 9.15, defecated — very watery. 12.00, midnight, hardly able to stand. Refused food. Senses dulled. Nose cold and moist. *April 10*, 8.30 A.M., nose dry and warm. Unable to rise. 8 P.M., remained in any unnatural position, however uncomfortable. 9.00, arose with difficulty to defecate — diarrhœa. Food refused. 10.15, profound stupor. *April 11.* 9 A.M., odor of telluride remarkably strong. Temperature very much lowered — extremities cold. 10.30, convulsive movements. Unable to rise. 12.15 P.M., breathing slow and deep for several hours. Fæces — watery and bluish-black (color doubtless due to tellurium from ingested compounds in previous experiment). 3.15, no control of movements. 5.10, brownish red vomit, with much mucus. Acid to litmus, none free; contained pepsin. 8.00, unable to move, even with mechanical stimulation. 8.30, reddish black urine, 110 c.c., containing coagulable proteid. 9.15, coma. 9.45, convulsions. 9.50, breathing intermittent. 9.55, convulsions; death.

Post-mortem. 10.15 P.M., garlic odor from abdominal cavity. Blood very black. Not laky. No crystalline forms found in blood, such as Rabuteau described. Kidneys very black in cortical layer. Heavy deposit of metallic tellurium about points of injection, and some pus. Intestines very much inflamed. Gastro-intestinal tract lined with metallic tellurium (from previously ingested compounds). Stomach contents deep red, alkaline; contained pepsin. Liver congested. No other lesions observed. Parts removed for analysis.

These results tend to show that subcutaneous injections of tellurium salts are followed essentially by the general effects noted after intravenous injections, especially by Rabuteau and Czapek and Weil, except that with subcutaneous injections the effects are much more gradual. Particularly noticeable in this experiment were general depression, weakening of the reflexes, increasing stupor, paralysis, coma, and convulsions preceding death from asphyxia.

2. **Distribution of tellurium.** — We determined quantitatively[1] the amounts of tellurium distributed to the various organs of the dog into which the tellurium tartrate had been injected,[2] with results agreeing

[1] By the method outlined on page 109.

[2] This same animal had previously ingested 0.7 gm. Na_2TeO_3 and 0.73 gm. $Te(C_4H_5O_6)_4$, in experiments 3 and 4, page 135. Most of this was vomited, however, and much that remained in the tract finally passed out with the fæces, or was held in the intestinal mucous membrane. The total quantity of $Te(C_4H_5O_6)_4$ injected under the skin was 1.45 gm. — containing approximately 0.31 gm. Te.

in the main with the qualitative conclusions drawn by previous observers. The figures given below show relative distribution, and they indicate that tellurium is readily soluble in the tissue fluids and, as Beyer has demonstrated histologically, may be carried to and deposited in almost all parts of the system:

	Te in mgs.
Muscle and skin about points of injection[1] (300 gms.)	38
Liver	12
Kidneys	9
Blood, clots from heart and large vessels (150 gms.)	8
Bile, 11 c.c.	7
Stomach	5
Urine, 110 c.c. (April 11)	4
Brain	4
Bladder	2
Stomach contents	2
Muscle, from shoulders and fore legs (150 gms.)	trace
Lungs, pancreas, spleen	trace

We see from the above results that the liver and kidneys contained a fairly large proportion of tellurium, and it is obvious that these organs have much to do with its separation from the blood and subsequent elimination. The comparatively large quantities in the urine and bile show this conclusively. In spite of the strong odor of the breath, the lungs contain at any one moment only traces of tellurium.

IV. Elimination of Tellurium.

Tellurium compounds appear to be quickly reduced after they enter the body. In all our feeding experiments the fæces contained much of the bluish-black metal, the walls of the gastro-intestinal tract were lined with reduced tellurium and even the material in the vomit — pieces of meat as well as mucus — showed reducing action by holding tellurium in metallic form. Consequently a great part of ingested tellurium is eliminated in metallic form with the intestinal excrementitious matter. When dosage was excessive, or when tellurium was introduced under the skin, appreciable quantities were eliminated in solution in the urine.[2] When the quantities carried into the stomach were small, only traces of tellurium appeared in the urine — frequently none could be detected. After subcutaneous

[1] Discoloration (bluish black) extended far beyond the limits of the excised tissue, so that much more tellurium was deposited near by.

[2] Identical results were obtained by Hansen, Czapek and Weil and Beyer. Also by Kletzinsky: Wiener medicinische Wochenschrift, 1858, viii, p 355.

injection we have found tellurium in the urine and in the bile — proof of the elimination of that substance from the body by both the liver and the kidneys. The glandular and tissue cells appear to reduce the bulk of soluble tellurium compounds coming in contact with them and to retain the metal, although, as Hofmeister and Beyer have shown, they form methyl telluride also — probably from the metal.

This reduction takes place very readily, in contact with any protoplasmic substance. We ourselves have observed it when tellurium compounds were brought in contact with fresh meat. Scheurlen and also Klett have lately shown that bacteria reduce tellurite to tellurium and that the bacterial cell is colored by the metal under such conditions, thus furnishing a very satisfactory indicator of reducing power on the part of these organisms. Hansen first referred to this process in explanation of the pigmentation of the glands and the contents of the gastro-intestinal tract. Hofmeister noted that the methyl synthesis and the process of reduction are entirely independent of each other, and that the latter may take place all over the body. Beyer, working by histological methods, observed that granular tellurium was deposited only in form-elements — in nerve and glandular cells, leucocytes and striated muscle particularly. Endothelium, unstriated muscle, nerve and connective tissue fibres, on the other hand, were found to have no affinity for tellurium.

The continuous evolution of methyl telluride in the breath (noted by practically all observers under all circumstances, and a symptom in all our experiments), implies transformation of deposited metal into soluble and diffusible form and subsequent transference to the lungs. This elimination, as we have seen, invariably continues so long after the last dosage of tellurium that *gradual transformation of deposited metal* seems to be a necessary deduction.[1] Tellurium in the form of methyl telluride is thrown from the body, not only by the lungs, but also with the epidermal excretions, in the fæces and intestinal gases, and may, as Neusser has pointed out, give special odor to eructations.

[1] Hofmeister has, in fact, proved this. He injected pulverized, chemically pure metallic tellurium, suspended in 0.7% NaCl solution, into the jugular veins of rabbits. At first there were no special symptoms. After 2–3 days, however, the odor of methyl telluride appeared in the expired air and continued to develop. In this way, also, much of the metal deposited under the skin in our subcutaneous injection experiment must have been slowly transformed (page 142).

V. Personal Experiences.

There are no cases of fatal tellurium poisoning on record, so far as we have been able to ascertain, although comparatively small quantities have been destructive of life in the lower animals. Comparatively few facts have been collected regarding the action of tellurium in the human system. Sir J. Simpson records a case[1] in which a student inadvertently swallowed a dose of tellurium, which was followed by the evolution of such a persistent odor that for the remainder of the session he had to sit apart from his fellow-students.

Berzelius[2] found hydrogen telluride more irritant in its action and more poisonous in effect than the corresponding compound of sulphur. Both he and Kölreuter[3] have reported that the oxides of tellurium, as well as a number of salts of telluric and tellurous acids, have a very unpleasant metallic taste resembling that of compounds of antimony,[4] and that some have a nauseating action and are strongly emetic. Wöhler, at the time of his discovery of ethyl telluride,[5] stated that it is very poisonous. At that time and subsequently, while engaged in his chemical researches on ethyl telluride, Wöhler observed that his sweat and breath took on an odor closely resembling that of the substance he was working with.[6] One night while perspiring very freely, the garlic odor in his sweat became so great that he himself could hardly bear it. It persisted in his breath for weeks. During seven successive days Hansen took a total of 0.34 gm. of potassium tellurite. Unusual sleepiness, oppression in the cardiac region, nausea and abundant salivation were the chief symptoms observed. At the end of the dosage period there was complete loss of appetite. The gastric symptoms did not disappear completely until after a lapse of two weeks. The characteristic odor of the breath continued seven weeks. Hansen was unable to separate any tellurium from his urine. An experiment on his friend Von Röder presented essentially the same results. Heeren[7] states that when

[1] Quoted from Blyth: Poisons, their effects and detection, 1885, p. 559.

[2] Th. Husemann und A. Husemann: Handbuch der Toxikologie, 1862, p. 773.

[3] L. Gmelin: Handbook of Chemistry (Watts), 1850, iv, pp. 398, 399, 402, 403. Also, *Ibid.*, 1856, x, p. 309, and Berzelius: Traité de chimie, 1846, ii, pp. 225, 230.

[4] See foot-note, page 148.

[5] Wöhler: Annalen der Chemie und Pharmacie, 1840, xxxv, p. 112.

[6] Quoted from Hansen's paper. Also referred to by Gorup-Besanez: Lehrbuch der physiologische Chemie, 1878, p. 552.

[7] Heeren: Chemisches Centralblatt, n. F., 1861, vi, p. 916.

compounds of ethyl and methyl tellurides are merely touched with the fingers their characteristic odor is carried to all parts of the body, the breath acquiring it, also, in a few days. In addition to the facts, already referred to in the experience of Reisert,[1] metallic taste, after ingestion of 0.015 of tellurous oxide, was observed in an hour and persisted for three days. We have already alluded to the clinical observations of Neusser, Pohorecki and Combemale and Dubiquet.[2]

We are highly favored in being permitted to present the following statement from Professor Victor Lenher in this connection.

Professor Lenher says, " My work with tellurium was largely from a metallurgical standpoint. I frequently had occasion to make large quantities of tellurium. The oxide is volatilized at high temperatures. In the process of fusion of the metal some of it escaped into the air and a considerable quantity was involuntarily inhaled into the lungs. Inhalation of the volatile tellurous oxide was accompanied by a distinctly metallic taste, and the breath and secretions from the skin quickly took on the characteristic garlic odor. In my own personal experience this disagreeable odor remained for months. In one case it persisted for about a year. When particularly large quantities of the oxide were inhaled, great depression and weakness followed. One day, after having fused metallic tellurium in the open air for several hours, I was so overcome by the influence of the volatile oxide that I lay on my bed to sleep for a little while, intending to arise shortly and resume my work; but I slept soundly for eighteen hours without awakening once during that time. Severe constipation followed the inhalation of the oxide and even purgatives, such as compound cathartic pills and Rochelle salt, failed to move the bowels for several days at a time and occasionally for a week. The inhaled oxide did not diminish intestinal putrefaction. The fæcal odors were stronger than normally and, besides, distinctly garlic. As the tellurium disappeared from the system a return to normal conditions was experienced and the odor of the expired air steadily diminished. A few days after my worst experience I analyzed a large quantity of the urine, but could not detect any tellurium in it. The fæces were not closely examined, but they were not blackened by metallic tellurium. After inhalation of fumes of the oxide I have frequently felt nauseated, although I have never vomited."

We ourselves have had no particularly toxic experiences, although

[1] Pages 130 and 131.

[2] Pages 105 and 131.

the following facts observed by Dr. Gies may not be without some interest: At the close of the first metabolism experiment (see footnote, page 111) Dr. Gies had occasion to make a journey of some length. He was very much surprised to learn that a pronounced alliaceous odor was observed not only in his breath but also in the excretions from the skin. This information was offered independently by several friends. It seems probable, therefore, that some of the tellurium, in the methyl compound breathed out by the dog, was inhaled by him and retained in his system and then was gradually eliminated in the same form. Dr. Gies is certain that he did not at any time come in personal contact with the oxide, but while stooping over the dog to hold the dish containing the weighed food — from five to ten minutes at a time twice a day for over two weeks — he breathed the eliminated telluride in relatively large quantities. These brief intervals of special inhalation were usually followed by drowsiness, and sometimes by nausea. Each symptom was, however, of short duration.

VI. Summary of Conclusions.

Non-toxic doses of tellurium (in the forms of oxide, tellurite, tartrate and tellurate) did not materially affect metabolism in dogs brought to a state of nitrogenous equilibrium even when dosage was continued for a week. These substances appeared to stimulate proteid catabolism only slightly. They increased somewhat the weight of dry matter in the fæces and diminished, in small degree, the absorption of fat. The urine was unaffected in volume, specific gravity and reaction, but became dark brown in color during the dosage periods.

Large doses retarded gastric digestion, induced violent vomiting, loss of appetite and somnolence. They caused, besides, inflammation and disintegration of the mucous membrane of the gastro-intestinal tract and also intestinal hemorrhage.

Introduced under the skin, tellurium (tartrate) caused restlessness, tremor, weakening of the reflexes, somnolence, diarrhœa, paralysis, unconsciousness, stoppage of respiration and death, in convulsions, from asphyxia. At the point of injection much of the tellurium was deposited in metallic form, but it was also distributed in large quantity to most of the organs and tissues.

Methyl telluride invariably appeared in the breath a few minutes after introduction into the system of even very small quantities of tellurium. It persisted for months after the last dosage. The odor

of this substance was also detected in the fæces and urine, about the viscera and in the epidermal excretions.

Secretion of mucus in the stomach and intestines was greatly stimulated by tellurium. Regurgitation of bile into the stomach was a frequent result. Tellurium compounds, even in small proportion, markedly arrested the secretion of acid in the gastric juice.

In the gastro-intestinal tract tellurium compounds were quickly reduced and the metal deposited in great part in, and on, the mucous membrane. Intestinal putrefaction did not appear to be influenced in any degree. The intestinal contents were pigmented by reduced tellurium and much of the ingested substance was eliminated in metallic form in the fæces.

The action of trypsin and pepsin outside the body was not very perceptibly diminished by quantities of tellurium compounds under 0.6 per cent. Zymolysis was almost unaffected in the presence of as much as 1.25 per cent of some of the salts. Ptyalin was more easily affected, even by the faintly alkaline tellurate. Trypsin appeared to be least sensitive to destructive influence, acting rapidly in the presence of even 2.5 per cent of tellurite.

Tellurium was eliminated in metallic form in the fæces; as methyl telluride in the breath, urine, fæces and epidermal excretions; in a soluble form, in small quantity, in the urine and in the bile.

The urine was colored brown to yellowish green after heavy dosage with tellurium compounds, but return to normal coloration was rapid after administration had been discontinued. Albumin and bile pigment, besides tellurium, were the abnormal constituents of the urine, found after subcutaneous injections. Toxic quantities given by the mouth caused the appearance of coagulable proteid but neither bile pigment nor sugar in the urine.

In man tellurous oxide taken into the lungs in fairly large quantity caused nausea, metallic taste, somnolence, depression and constipation. Methyl telluride was excreted in the breath, through the skin and with the fæces. Inhalations of methyl telluride induced sleepiness and nausea and the breath and the excretions from the skin under these circumstances acquired, and retained for a long time, the odor of that substance.

In many respects the action of tellurium in the body is like that of selenium, arsenic and antimony.[1]

[1] Czapek and Weil have come to the same conclusion. It is interesting to note, in this connection, that tellurium is believed by some chemists to be in

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References in which only casual mention of effects of tellurium appear are given in the footnotes throughout this paper, pages 105, 131, 143, 145, and 146.

reality a mixture of elements, containing an antimony, arsenic-like body. Brauner calls one of the presumed constituents of the tellurium complex, *austriacum*, which may be the *dwitellurium* predicted by Mendeléeff. See Brauner: Journal of the Chemical Society (London), Trans., 1889, lv, p. 382, and Grünwald: *Ibid.*, Abstracts, 1890, lviii, p. 434; also, Dictionary of applied chemistry, Thorpe, 1893, iii, under "Tellurium." (See footnote, p. 105.)

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

THIRTEENTH ANNUAL MEETING.

JOHNS HOPKINS UNIVERSITY, DECEMBER 27 and 28, 1900.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY.

OBSERVATIONS ON THE BLOOD-PRESSURE DURING THE PERIOD OF NORMAL SLEEP.

C. E. BRUSH AND R. FAYERWEATHER (PRESENTED BY W. H. HOWELL).

THE paper described a series of experiments made with a modification of Mosso's sphygmomanometer to determine the curve of blood-pressure in man during a period of normal sleep. The instrument used was adapted to take the pressure in the large arteries of the wrist and hand, the mean pressure being determined by the maximum amplitude of the pulse waves. The results of the experiments show that general blood-pressure falls during sleep, reaching its lowest point about one hour after sleep has begun. From this point onward during a period of sleep of from six to seven hours the blood-pressure rises gradually until the period of awaking. The rise in pressure, although continuous on the whole, is broken by variations which occur with some regularity and present the general character of periodic waves. The curves obtained indicate that a relaxation of the arteries occurs up to the point of deepest sleep, and that subsequently an increasing vaso-constriction takes place, which culminates at the time of awaking.

A NOTE ON THE USE OF A SATURATED SOLUTION OF MAGNESIUM SULPHATE FOR PREVENTING THE COAGULATION OF BLOOD IN BLOOD-PRESSURE EXPERIMENTS.

W. S. CARTER (PRESENTED BY W. H. HOWELL).

THE paper called attention to the serious danger that may arise, particularly in pharmacological experiments, from the use of solutions of magnesium sulphate for the purpose indicated in the title of the paper. Any marked fall of pressure during such an experiment may result in the entrance of some of the magnesium sulphate into the circulation, and in the smaller animals at least this occurrence may result in death.

SOME NEW OBSERVATIONS ON BLOOD-PLATES AND LEUCOCYTES.

By GEORGE T. KEMP and HENRIETTA CALHOUN.

The number of red corpuscles was determined by the hæmatocrit, and the plates and corpuscles were counted in a Thoma-Zeiss hæmocytometer. A drop of the "fixative" was placed on the finger and the blood received from the vessel directed into it.

The following fluid is recommended as a fixative: —

Formic aldehyde 40% solution . . .	1 part.
Sodium chloride 1% solution . . .	15 parts.
Methyl green, enough to color.	

The fluid therefore contains $2\frac{1}{2}$ per cent formic aldehyde, and a trifle less than one per cent sodium chloride. This fixes the histological elements of the blood rapidly, preserves their form well, and colors the blood-plates and leucocytes distinctly.

The average ratio of blood-plates to red corpuscles was found to be for men, 1 : 5.8; for women, 1 : 5.4. These figures are the result of fourteen different counts of specimens from eleven men, and of six different counts of specimens from two women. Taking the normal number of red corpuscles to be 5,000,000 for men and 4,500,000 for women, the count of blood-plates, to the cubic millimetre, would be for men, 862,000, for women, 833,000. There was a marked tendency for the number of blood-plates to increase and diminish with the number of corpuscles.

The leucocytes do not break down during normal coagulation of the blood, while the blood-plates do break down.

ON THE RATE OF FATIGUE OF NERVE CENTRES.

By R. S. WOODWORTH.

If a muscle is more rapidly fatigued when excited by its nerve centre than when excited by stimuli applied to its motor nerve, the difference in rate will represent the rate of central fatigue. On trial, no difference is found. A frog's muscle excited reflexly fatigues at sensibly the same rate as its companion muscle stimulated directly. Human muscles, so far as tested, fatigue no more rapidly on voluntary than on electrical stimulation. In these cases, then, no sign of central fatigue is detected.

Another method consists in retarding or removing fatigue by acting on the centres or on the peripheral organs. If, in ergographic work, the flexors are completely relaxed and even stretched by the action of their antagonists between successive contractions, about one-half of the fatigue is prevented, and a series of over a thousand contractions may be made with a loss of less than 10 per cent of the initial force. When, on the contrary, the contraction is sustained throughout, the loss is much more rapid. This rapid fatigue of sustained contraction has been supposed by Treves to be of central origin, but it is undoubtedly an effect of the extreme contracture, since it is largely removed by interpolating a short series of alternate contractions and relaxations. That this recovery from fatigue (and therefore the fatigue itself) is of muscular origin is indicated by the fact that the same result can be obtained from an isolated frog's muscle.

Still another way of searching for central fatigue is to consider cases in which a nerve centre works hard without producing strong muscular contraction, and hence without causing much muscular fatigue. Experiments on the accuracy of a movement have this character, as have also various mental acts. It is found that the same movement or mental act can be repeated constantly with maximal effort for hundreds and thousands of times, and with slight loss of efficiency.

It seems reasonable to conclude that nerve centres fatigue very slowly. In fact we have no experimental proof that they fatigue at all.

FURTHER OBSERVATIONS ON EPINEPHRIN.

By JOHN J. ABEL.

NEW methods of preparing this compound which do not involve benzoating or hydrolytic treatment in the autoclave have shown that some of its properties as heretofore described are not inherent in the native substance, but are developed by manipulation. Thus, as v. Fürth has found, the active principle of the adrenal gland not subjected to treatment in the autoclave is not precipitated by ammonia and fails to respond to a number of alkaloidal tests. These properties are, however, readily brought out by heating in the autoclave with a little dilute sulphuric acid for a short time under a pressure of four or five atmospheres. After the autoclave treatment

an iron compound may be formed directly from the aqueous solution; this compound, however, is physiologically inactive, differing in this respect from the iron compound made in methylic alcohol solution.

In a word, "suprarenin" is only a modification of epinephrin, being the native principle not subjected to benzoating or treatment in the autoclave.

From the iron compound of "suprarenin" it is easy to make the benzoyl compound of epinephrin and from this in turn the entire series of derivatives formerly described by me.

The salt which I have called epinephrin bisulphate is quantitatively convertible into an iron compound. Fractional precipitation with ammonia also shows that it contains no other substance than epinephrin. Analyses now being made will show what changes take place in the autoclave and what relation exists between the autoclave products and the less manipulated, more highly active compounds recently isolated by myself and others. Experiments on the blood-pressure of animals appear to show that the physiological activity of the compound is greatly lowered by this treatment in the autoclave. Statements previously made as to the ease with which inactive modifications of epinephrin are produced have been fully substantiated by later work.

FURTHER OBSERVATIONS ON THE BLOOD-PRESSURE-LOWERING BODIES IN EXTRACTS OF THE SUPRARENAL GLAND.

By REID HUNT.

Evidence was obtained that aqueous extracts of the suprarenal contain at least one other blood-pressure-lowering body in addition to choline, the presence of which in the extracts of the suprarenals causes a fall of blood-pressure. This second body differs from choline in its physiological effects chiefly in that it causes a fall of blood-pressure after the administration of atropine. It also seems to be more toxic. By repeated precipitation with platinum or mercuric chloride, or by treating the filtrates from these precipitates with hydrogen sulphide, this second body disappeared; at the same time the amount of choline seemed to be increased. Hence it was considered probable that some body was present which yielded choline on decomposition. That this body was not lecithin or jecorin

was shown by the fact that its solubilities differed from theirs. It was suggested that this "precursor" of choline might be some ester-like body containing choline in its molecule. A number of such esters are known to chemists, but their physiological action has not been tested.

It was found that a distinct fall of blood-pressure could be obtained when a solution containing but three hundredths of a milligramme (per kilogramme of animal) was injected into the vein of a dog. It is not claimed that this blood-pressure-lowering body has been isolated in even an approximately pure condition.

Results similar to the above were obtained in a few experiments with extracts of the brain.

ON THE EFFECTS OF INTRAVENOUS INJECTIONS OF MINIMAL DOSES OF EPINEPHRIN SULPHATE UPON THE ARTERIAL BLOOD-PRESSURE.

By REID HUNT.

Curves were exhibited demonstrating the effects obtained in dogs by the injection of Abel's active epinephrin sulphate in doses varying from 0.083 to 5.7 millionths of a gram per kilogramme of body weight. The vagi were previously paralyzed by atropine. The injections were made rapidly, their duration varying from two to four or five seconds. Such results as the following were constantly obtained.

	Rise of blood-pressure.
0.083 millionths of a gram per kilo body weight	5 mm. Hg.
0.23 millionths of a gram per kilo body weight	7 mm. Hg.
0.49 millionths of a gram per kilo body weight	15 mm. Hg.
0.69 millionths of a gram per kilo body weight	20 mm. Hg.
1.7 millionths of a gram per kilo body weight	24 mm. Hg.
5.7 millionths of a gram per kilo body weight	66 mm. Hg.

These results show that epinephrin sulphate is many times more powerful physiologically than the aqueous extracts of the medulla of the suprarenal obtained by Moore and Purinton.

The rise of blood-pressure was, at times, followed by a fall of blood-pressure; the latter was more marked as a rule with the larger than with the smaller doses. In many cases only a rise of blood-pressure followed the injection of minimal doses. Control experiments with distilled water and normal saline solution showed that the rise of pressure was not due to the volume of liquid injected.

Curves were exhibited showing the difference between the effects of the rapid and slow injection of solutions of epinephrin sulphate. In one case, for example, 2.2 millionths of a gram per kilo body weight, injected in the course of forty seconds, caused a rise of but 8 millimetres of mercury, whereas immediately afterwards one half of this dose (1.1 millionths of a gram per kilo), injected rapidly, caused a rise of 14 millimetres.

These experiments show that Abel's claim that epinephrin is the blood-pressure-raising constituent of the suprarenal is not impaired by objections based upon the alleged greater activity of so-called crude extracts.

ANALYSIS OF SOME NUCLEIC ACIDS.

By P. A. LEVENE.

THE paranucleic acids of vitellin and of the ichtulin of the cod-fish egg were found to contain:

	C	H	N	P
Vitellinic acid . .	32.31	5.58	13.13	9.88
Ichtulinic acid . .	32.56	6.00	14.00	10.34

Thus these "paranucleic" acids of different origin have a comparatively similar composition. The difference in the nitrogen is easily explained by the fact that the ichtulinic acid was obtained from the ammonium salt, and the vitellinic from the copper salt. If the latter acid is obtained from the ammonium salt it also contains about 14 per cent of nitrogen.

The nucleic acids analyzed were those of the pancreas of the cod-fish, the sperm of the codfish, and the Bacillus tuberculosis.

	C	H	N	P_2O_5
Pancreas	36.50	4.69	16.70	20.16
Cod-fish sperm	36.73	5.12	16.78	20.47
Bacillus tuberculosis . . .	33.78	6.32	9.42	29.40

The acid obtained from the pancreas in distinction from the guanilic acid described by Bang contains in its molecule adenin in addition to guanin. This acid, as well as that of the cod-fish sperm, does not differ much in its composition from the acids obtained from different sources[1] within the last year by Schmiedeberg, Herlant, and Osborne.

[1] The only acid which differs considerably from the rest is that obtained from bacteria.

On precipitating the nucleic acids directly from the tissues, glycogen is precipitated simultaneously. The two can be separated by means of copper chloride. The nucleic acid forms a copper salt insoluble in water, while the copper compound of glycogen is soluble. By this method glycogen may be obtained from the pancreas, and a glycogen-like substance from the Bacillus tuberculosis.

PHYSIOLOGICAL STUDIES OF THE BLOOD TAKEN FROM ANIMALS DEPRIVED OF THE ADRENALS.

By I. LEVIN.

In order to determine whether the function of the suprarenals consists in some antitoxic influence on the blood, a tracing of the blood-pressure was taken from a dog while blood of a normal dog was injected into the veins. The intravenous injection of normal blood did not in any way change the character of the blood-pressure tracing. The same experiments were repeated, with the sole difference that the blood for the intravenous injection was taken from dogs whose adrenals had been extirpated five hours previous to the bleeding. The intravenous injection always produced a marked rise of the blood-pressure.

The relative duration of systole and diastole of the heart was changed by the poison.

The conclusion to be drawn is that the blood of an animal deprived of the adrenals contains substances which act upon the blood-pressure, but that these substances do not exist in the blood of a normal animal. They must therefore be neutralized by the adrenals.

FURTHER EXPERIMENTS ON THE EXCRETION OF KYNURENIC ACID.

By LAFAYETTE B. MENDEL and E. C. SCHNEIDER.

The investigations of Jaffé and his co-workers on the production of kynurenic acid in the dog have generally been accepted as indicative of its intestinal origin. The observations which have occasioned such conclusions are to be found in experiments (by Haagen, Rosenhain, and Josephson) in which the administration of substances known to diminish intestinal putrefaction was followed by a marked decrease in kynurenic acid excretion. Not all intestinal antiseptics, however,

were productive of such results. Mendel and Jackson[1] have reviewed the evidence presented by previous investigators and have published the results of a series of experiments which indicate that kynurenic acid is a direct product of proteid catabolism, and is in large part, if not entirely, independent of putrefactive changes in the intestine. The experiments now reported form a confirmation of this previous work. Thus with six dogs, each of which had previously fasted six days or more, a marked excretion of kynurenic acid was still observed at the end of the fasting period. Even more conclusive are the results obtained with dogs that fasted and at the same time received large doses of calomel. The complete disappearance of ethereal sulphates from the urine in every case gave satisfactory indication of the absence of putrefactive processes in the intestine; under these conditions kynurenic acid was nevertheless excreted in amounts varying from 12 to 158 milligrams per day by every one of the animals. Further observations on the rôle of various foodstuffs in kynurenic acid production may here be briefly summarized. In confirmation of Mendel and Jackson's experiments we have failed to obtain any output of kynurenic acid after gelatin feeding. The same experience followed ingestion of ovomucoid, elastin, so-called "chondrin" obtained by the hydration of cartilage, and commercial thymus powder. Experiments still in progress were reported regarding the influence of various cleavage and digestion products of proteids on the elaboration of kynurenic acid by the animal economy.

DOES MUSCLE CONTAIN MUCIN?

By G. A. FRIED and WILLIAM J. GIES (Reported by W. J. GIES).

With a view of testing the work which led to disagreement between Schepilewsky and Goodman, the connective tissue residues from 3–5 lbs. of beef and veal, prepared by Schepilewsky's method, were extracted in the usual manner in half saturated lime or baryta water. (Muscle fibres could never be completely removed before the extraction.) Seven such extractions were made with as many samples of fresh muscle in appropriate quantities of dilute alkali. On neutralization, and weak acidification, with 0.2 per cent HCl, a heavy precipitate was obtained in each extract, but the substance so precipitated quickly

[1] Mendel and Jackson: This journal, 1900, iii, p. iii.

dissolved each time in slight excess of acid (alkali albuminate?). In this respect its behavior was very different from that of connective tissue glucoproteid. Only a faint turbidity suggested traces of mucin. In one experiment, in which Goodman's procedure was somewhat altered, the connective tissue residue obtained by Schepilewsky's method was treated first with half saturated lime-water, and later with 5 per cent KOH. On rendering the extract only very faintly acid a proteid precipitate was obtained in each case. This was filtered off, purified and analyzed. With another portion of tissue half saturated baryta water and subsequently 5 per cent NaOH were used with the same result. The average nitrogen content of the ash-free substance obtained from each extract was as follows: —

1. $Ca(OH)_2$, 16.39%. KOH, 15.12%.
2. $Ba(OH)_2$, 16.69%. NaOH, 14.84%.

None of these preparations yielded reducing substance on decomposition with acid. We are strongly inclined to the belief that these products are alkali albuminate, or at least are admixed with the same. They are neither the "stroma substance" of Goodman nor the mucin of Schepilewsky. Schepilewsky's method will not detect very small quantities of mucin.

METHODS OF PREPARING ELASTIN, WITH SOME FACTS REGARDING LIGAMENT MUCIN.

By A. N. RICHARDS and WILLIAM J. GIES (Reported by W. J. GIES).

In continuation of the studies reported at the previous session of the Physiological Society, we find that the ligamentum nuchæ of the ox contains an appreciable quantity of mucin, having all the qualities of the glucoproteids separable from white fibrous connective tissue. The nitrogen of five different preparations varied from 12.90 per cent to 13.86 per cent, the sulphur from 1.32 per cent to 2.05 per cent.

In order to insure removal of mucin and coagulable proteids from ligament in the preparation of elastin, we have extracted the finely divided tissue for several days in large excess of cold half saturated lime-water. This preliminary process makes extraction of the tissue with hot alkali unnecessary, and thereafter, when the usual method is continued, neither albumin nor globulin is present to be coagulated and there is no mucin to be decomposed.

By this improved method we have made three different preparations of elastin from the ligamentum nuchæ of the ox. Each

contains less sulphur than elastin obtained by the old method, the quantity varying from 0.13 to 0.17 per cent (not deducting S of the ash, amounting to 0.11 per cent of the purified substance). We have observed in two preliminary experiments that all the sulphur in the elastin prepared by our own method is firmly united in the elastin molecule and is not broken away on boiling with 1 per cent KOH. This result is not obtained with elastin prepared by the older method, in which extraction with alkali is avoided.

Using Schultze's method, the distribution of nitrogen in the elastin prepared by the improved process as contrasted with that of the old was found to be as follows: —

	Ammonia.	Bases.	Amido acids.	Total percentage.
A Old method (1)	2.26	2.98	95.44	100.68
(2)	2.34	2.26	98.42	103.02
B Improved method.	1.73	3.08	95.23	100.05

Our results in this connection seem to indicate that elastin does yield organic bases, as Kossel and Kutscher have contended in opposition to Bergh and Hedin.

ON THE OCCURRENCE OF LIPASE IN THE BODY, AND ITS REVERSIBLE ACTION.

By A. S. LOEVENHART.

STRAINED extracts of many tissues were prepared and their lipolytic activity studied as follows: —

4 c.c. H_2O, 1 c.c. extract, and 0.1 c.c. toluene (antiseptic) were heated at 40° for 5 minutes. 0.26 c.c. ethyl butyrate was then added. After heating 15 min. at 40° the butyric acid produced was titrated with $\frac{N}{20}$ KOH using litmus as the indicator. The following are the relative lipolytic activities found for the organs of the pig.

Pancreas	1.0
Liver	2.93
Intestinal mucosa	0.75
Kidney	0.50
Submaxillary	0.36

The mucosa of the stomach was found to possess slight but distinct activity.

Kastle and Loevenhart[1] succeeded in showing that lipase acts reversibly on ethyl butyrate, being capable of decomposing it into butyric acid and ethyl alcohol, and also being capable of synthesizing ethyl butyrate from these products. This result can be applied qualitatively to fats and other ethereal salts. In the present investigation it was sought to ascertain to what extent the fat syntheses occurring in the body could be due to lipase. With this in view the occurrence of the enzyme in the body has been studied in some detail. All tissues tested have been found to possess more or less lipolytic activity. Lipase has been found in the following localities: liver, pancreas, intestinal mucosa, kidney, submaxillary, gastric mucosa, active mammary gland, lung, brain, lymphatic glands, subcutaneous fat (all fat deposits), lymph, spleen.

The degree of lipolytic activity possessed by an organ seems to be related to the fat transformation, whether synthetic or destructive, known to occur in the organ. Thus the active mammary gland has been found to possess great lipolytic activity, equal to that of the pancreas, while the resting mammary gland possesses but a trace of activity. The work of Kastle and Loevenhart shows that lipase merely has the power of establishing equilibrium between ethyl butyrate, butyric acid, and ethyl alcohol. This fact together with the occurrence of lipase in considerable quantities in subcutaneous fat offers an explanation of the storing of fat in this locality and it also explains the absorption of this fat during inanition or malnutrition. In these states the blood and lymph may be supposed to become poor in fatty acid and glycerine, in which case the lipase in the subcutaneous fat would tend to restore equilibrium by effecting the hydrolysis of fat. These results throw light on the phenomena of fat absorption.

OBSERVATIONS ON THE PRODUCTS OF PAPAIN AND BROMELIN PROTEOLYSIS.

By LAFAYETTE B. MENDEL and F. P. UNDERHILL, Ph.B.

A review of the literature regarding the proteolytic enzymes of vegetable origin reveals a lack of sufficient knowledge to enable us to tell with certainty whether only one or several types exist. In many instances the only data at present available are observations regarding the conditions under which proteolysis may proceed; the

[1] Kastle and Loevenhart: American chemical journal, 1900, pp. 24 and 491.

nature of the products resulting from the action of the known vegetable enzymes has been considered only to a very limited extent. Papain (also called papayotin) — the proteolytic enzyme of the Carica papaya — has usually been regarded as closely related in action to the trypsin of the pancreas. It dissolves proteids in alkaline, neutral or even slightly acid media, and a basis for a comparison with trypsin is thus indicated. There are observations on record indicating not only the formation of proteoses and peptone, but also of leucin and tyrosin. The statements regarding the occurrence of these two amido-acids are, however, exceedingly meagre, and tyrosin has occasionally been missed altogether. Our own experiments on papain proteolysis have all been made with commercial preparations of various origins. Proteolytic action was studied in different media and on several proteids, such as fibrin, egg-albumin, casein, etc. Careful control experiments were made and various antiseptics (mainly NaF) were tried. The more important results of these observations may be summarized briefly as follows: (1) The papain preparations show proteolytic activity in both alkaline and slightly acid media. (2) The primary products of proteolysis, carefully studied in the case of casein, correspond closely with those obtained by F. Alexander from peptic digestions. (3) Leucin, tyrosin, and tryptophan (proteinochromogen) — all characteristic products of tryptic proteolysis — were not detected in any digestion where the influence of bacteria or bacterial enzymes was excluded. (4) These observations indicate that papain differs from known proteolytic enzymes of animal origin, and also from vegetable enzymes like the bromelin (from the pineapple) which readily forms leucin, tyrosin, and tryptophan, even in acid media.

CHANGES IN THE COMPOSITION OF THE COCOANUT DURING GERMINATION.

By J. E. KIRKWOOD and WILLIAM J. GIES (Presented by W. J. GIES).

The fresh nuts in the husk were placed on earth kept constantly moist at a tropical temperature. After a period of about four months the shoots appeared through the husk. At the end of a year of germination chemical examination was begun. At this time the milk cavity of the ovule was completely filled with the fully developed cotyledon, which had almost entirely absorbed the endosperm at the "stem end," and considerably thinned it posteriorly.

The cotyledon, particularly the central, more vascular portion, contains considerable diastatic ferment, and apparently, also, a trace of proteolytic enzyme. Cellulose-dissolving and fat-splitting enzymes have, however, not yet been detected. The appended table presents a few of our analytic results in percentage figures, showing the distribution of water, solids, inorganic matter, and nitrogen, from which numerous deductions as to general growth may be readily drawn: —

			Water. %	Solids. %	Inorganic matter. %	Nitrogen. %
A	Roots.	Tips	89.89	10.11	1.33	
		Tips to husk	86.41	13.59	1.57	
		Very near husk	82.79	17.21	1.60	
		Inside of husk	77.92	22.08	1.20	0.27
B	Stem.	"Root crown"	86.21	13.79	1.05	0.53
	Petioles		83.63	16.37	1.43	0.29
	Leaves.	Young	74.66	25.34	1.65	
		Old	71.99	28.01	1.90	0.45
C	Cotyledon.	"Neck"	78.98	21.02	1.42	
		Cortex	80.83	19.17	1.74	0.31
		"Heart"	88.99	11.01	0.78	0.14
D	Endosperm.	Anterior	23.42	76.58	0.84	
		Posterior	46.08	53.92	0.98	0.65
E	Ungerminated nut.	Endosperm	46.00	54.00	1.03	0.75
		Milk	95.30	4.70	0.50	

A FURTHER STUDY OF THE GLUCOPROTEID IN BONE.

By P. B. HAWK and WILLIAM J. GIES (Presented by W. J. GIES).

Five different preparations from the femur of the ox have been analyzed since the figures for the first two (from rib and femur of the ox) were reported to this Society a year ago. The elementary composition of the seven vary between the extremes here given in percentage figures: —

C	H	N	S	Ash
45.75—48.08	6.66—7.29	11.97—14.15	1.36—2.21	0.33—2.72

The ash-free substance does not contain phosphorus. The amount of sulphur that could be split off in the form of ethereal sulphate varied from 0.49 to 1.10 per cent.

The following figures show the average percentage composition of the preparation of osseomucoid, which we have good reason to think is the purest, and also of chondromucoid, as determined by Mörner: —

	C	H	N	S	O	S (as eth. sulph.)
Osseomucoid . .	46.41	6.76	12.08	2.04	32.71	1.08
Chondromucoid .	47.30	6.42	12.58	2.42	31.28	1.72

Compared with the glucoproteid of cartilage, osseomucoid appears to contain more hydrogen and oxygen and correspondingly less of the other elements. In its reactions it is practically the same.

THE EFFECT OF CARBON DIOXIDE AND OXYGEN ON SMOOTH MUSCLE.

By ALLEN CLEGHORN, assisted by H. D. LLOYD.

A ring of smooth muscle, made by two parallel sections across the stomach of a frog, including the mucous membrane, was suspended from a metal electrode in a moist gas chamber; a second electrode attached the muscle to the writing lever.

1. On passing carbon dioxide through the gas chamber an almost immediate increase in the tonus of the muscle took place.

2. When this increase in the tonus was considerable, electrical make currents had no effect, but break currents often caused a fall.

3. While the muscle was contracting spontaneously carbon dioxide caused a rise in tonus, but eventually stopped the contractions.

4. Electrical stimulation while the muscle was under the influence of carbon dioxide was followed by long and slow contraction.

5. Oxygen gave no pronounced effect during spontaneous or stimulated contraction. Sometimes the contractions appeared larger but less frequent.

6. Often the break current caused a larger contraction than the make; carbon dioxide would then reverse the result.

7. Carbon dioxide prolonged the latent period considerably.

8. When spontaneous contractions of the muscle were brought to a standstill by carbon dioxide, recovery did not seem to be accelerated by the application of oxygen.

In order to guard against errors from local polarization at the metal electrodes the experiments were repeated with non-polarizable electrodes. The same results were obtained.

On the Movements of the Œsophagus and the Cardia. By S. J. Meltzer.

On some of the Complexities of the Centre of Deglutition. By S. J. Meltzer.

A Student's Laboratory Table. By E. T. Reichert.

A New Rheotome. By E. T. Reichert.

A Rheocord. By E. T. Reichert.

A Rheocord. By G. P. Clark.

A Hair-cast of a Living Human Stomach. By G. P. Clark for Dr. Nathan Jacobson.

On the Oxidation of Native Pigments. By Walter Jones and John Auer.

A Study of the Seasonal Variations of Growth in Weight of Children. By G. W. Fitz and F. W. Hutchings.

Read by title.

The Physiological Action of Three Poisonous Mushrooms — Amanita Muscaria, Amanita Bulbosa or Verna, and Amanita Phalloides. By W. S. Carter.

Read by title.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. E. L. MARK, DIRECTOR. No. 120.

THE DIRECTIVE INFLUENCE OF LIGHT ON THE EARTHWORM ALLOLOBOPHORA FŒTIDA (SAV.).

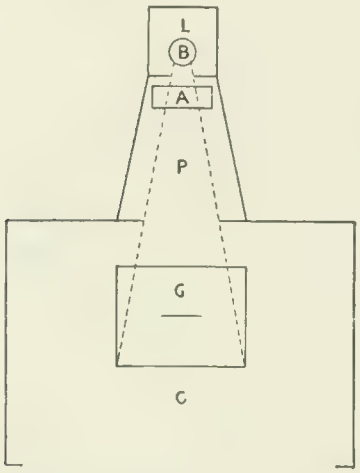
BY G. H. PARKER AND L. ARKIN.

WHEN earthworms are exposed to light of moderate intensity, they usually creep away from its source. Both Hoffmeister ('45, p. 18) and Darwin (:00, p. 19), who recognized the eyeless condition of these animals, believed this to be due to the stimulating effect of light on the anterior end of the worm, but Graber ('84, p. 290) observed similar reactions in worms from which the anterior segments had been cut off and thus demonstrated that other parts than the head were stimulated by light. Graber's observations were confirmed by Yung ('92), and later by Hesse ('96), who also showed, by putting earthworms in glass tubes over which movable opaque cylinders were slipped, that every part of the exterior of the worm was sensitive to light.

The present investigation was made to ascertain the influence of light on the *direction* in which one of the common earthworms, Allolobophora fœtida (Sav.) moves, and to find through which of the external regions of its body this directive influence is exerted.

The experiments were carried on in a dark chamber, a ground plan of which is given in the accompanying figure. This chamber (C) was illuminated by light from a Welsbach gas burner (B), contained in a small lantern (L). The light emerged from the lantern through a vertical slot 10 cm. high and 3 cm. broad, and passed through a light-proof passageway (P) to the dark chamber. In this passageway near the burner was set a glass vessel (A) having flat, parallel sides 4 cm. apart and containing a saturated solution of alum for the interception of heat rays. A large horizontal beam of light entered the dark chamber through an opening in its side 15 cm. square. Directly in the path of the light was suspended from the roof of the chamber a horizontal glass plate (G) about 20 cm. broad and 25 cm. long. This plate was hung by cords which were attached to its four corners and met at a single point of

support near the roof of the chamber. Thus the plate, though firmly suspended, could be easily rotated about its vertical axis. The upper surface of the plate was covered with a sheet of wet filter paper, and on this the worms to be experimented upon were allowed to creep. The light employed was a 16-candle Welsbach gas burner. With use its intensity diminished till at the end of the experiments it measured 13.5 candle power. The experiments were therefore assumably performed with a light of from 14 to 15 candle power. The distance from the light to the centre of the plate on which the worms were placed was exactly 50 cm.



Ground plan of dark chamber, × $\frac{1}{20}$. A, glass containing saturated aqueous solution of alum; B, Welsbach gas burner; C, dark chamber; G, glass plate, the centre of which is 50 cm. from the front face of the Welsbach burner; L, lantern; P, light-proof passageway.

Four sets of experiments were tried and in each set ten worms were used. Each worm was made to creep across the plate in a direction at right angles to the rays of light. If in creeping the worm turned either toward the light or away from it, the plate was rotated so that the chief axis of the worm was kept in uniform relation to the light. To form some estimate of the directive action of the light, the locomotor movements of the worm were carefully observed. Each movement was initiated by projecting the head. The direction which the head takes determines the direction in which the rest of the worm will ultimately move, and this can be used as a means of estimating the directive action of the light. Three classes of head movements can easily be distinguished: first, a movement directly forward on the line previously indicated by the animal; second, one away from the light; and third, a movement toward the light. For each worm the directions of fifty head movements were observed and recorded, twenty-five movements with the light on the right and twenty-five with it on the left side of the animal. Thus each of the four sets of experiments includes fifty observations on each of ten worms, or five hundred observations in each set.

In each of the four sets of experiments a different part of the worm was exposed to light. In the first set the whole length of the worm was illuminated. In the second, by narrowing the opening

into the dark chamber and moving the glass plate sidewise as the worm crept over it, the light was limited to the anterior third of the worm. In the third set, by similar devices, the middle third of the worm was illuminated, and in the fourth the posterior third.

Table I gives the results of the first set of experiments, in which the whole length of the worm was illuminated, first on the right side (upper section of table) and next on the left (lower section). The

TABLE I.

Light falling upon *whole length* of worm.

No. of the worm.		1	2	3	4	5	6	7	8	9	10	Total.
Light on right side of worm.	Indifferent	15	16	16	18	19	12	20	16	23	9	164
	Away	10	8	8	7	6	11	5	7	1	12	75
	Toward	0	1	1	0	0	2	0	2	1	4	11
Light on left side of worm.	Indifferent	11	19	17	13	17	14	18	20	15	20	164
	Away	12	5	8	8	8	10	6	5	10	4	76
	Toward	2	1	0	4	0	1	1	0	0	1	10

individual worms used are designated at the top of the table by numbers from 1 to 10. Under each of these in the two principal sections of the table are given the number of times, in a total of 25, that the worm moved its head directly forward (*indifferent* to the light), *away* from the light, or *toward* the light. Although individual worms gave somewhat different results according to the side of the body illuminated, the total results for the two sides, as shown at the end of the table, are remarkably uniform. Combining these results, it appears that in five hundred head movements three hundred and twenty-eight (65.6%) were directly forward, one hundred and fifty-one (30.2%) away from the light, and twenty-one (4.2%) toward the light. When a specimen of Allolobophora fœtida is set creeping across a plate at right angles to the direction of the light and the plate is kept stationary, the worm gradually turns its anterior end away from the light and finally creeps in an almost straight line with the light behind it. Thus Allolobophora fœtida, like other species of earthworms, is negatively phototactic, and of the five hundred head movements previously recorded, the twenty-one toward the light

must, therefore, have been due to other stimuli than light. Such stimuli are doubtless to be found in the slight roughness of the paper, etc., and would tend to turn the worm as often away from the light as toward it. Hence it follows that a part of the deflections from the light (twenty-one in number) were probably due to other causes and that consequently the real deflecting effect of the light is not to be estimated at one hundred and fifty-one in five hundred but at that number less twenty-one, or one hundred and thirty (26%). It thus appears that under the conditions of the experiments 26% is a measure of the deflecting effect of the light.

The results of a similar series of experiments, in which, however, only the anterior third of the worm was illuminated, are shown in Table II. Here it will be observed that of five hundred head move-

TABLE II.

Light falling on *anterior third* of worm only.

No. of the worm.		1	2	3	4	5	6	7	8	9	10	Total.
Light on right side of worm.	Indifferent	7	11	19	12	12	13	13	17	12	23	139
	Away	13	8	4	8	10	5	7	5	5	2	67
	Toward	5	6	2	5	3	7	5	3	8	0	44
Light on left side of worm.	Indifferent	10	10	17	16	5	12	11	19	15	19	134
	Away	10	9	6	7	11	7	8	5	6	3	72
	Toward	5	6	2	2	9	6	6	1	4	3	44

ments two hundred and seventy-three (54.6%) were directly forward, one hundred and thirty-nine (27.8%) were away from the light, and eighty-eight (17.6%) toward it, leaving a residue of fifty-one (10.2%) to be ascribed to the directive influence of the light, a considerable reduction as compared with the measure obtained from the action of the light on the whole length of the worm.

When the light is limited to the middle third of the worm, the reduction of its directive influence, as shown in Table III, is still greater.

In five hundred head movements three hundred and thirty-six (67.2%) were directed forward, eighty-eight (17.6%) away from the

light, and seventy-six (15.2%) toward it, leaving only twelve (2.4%) of the movements to be accounted for by the directive action of the light.

TABLE III.

Light falling on *middle third* of worm only.

No. of the worm.		1	2	3	4	5	6	7	8	9	10	Total.
Light on right side of worm.	Indifferent	7	10	19	19	12	21	22	17	24	15	166
	Away	10	8	3	3	7	2	2	5	1	4	45
	Toward	8	7	3	3	6	2	1	3	0	6	39
Light on left side of worm.	Indifferent	9	13	18	18	14	19	20	19	23	17	170
	Away	10	8	4	3	4	2	3	3	1	5	43
	Toward	6	4	3	4	7	4	2	3	1	3	37

Finally, when the light is restricted to the posterior third of the worm, the directive influence, as shown in Table IV, almost entirely disappears. For in five hundred head movements three hundred and

TABLE IV.

Light falling on *posterior third* of worm only.

No. of the worm.		1	2	3	4	5	6	7	8	9	10	Total.
Light on right side of worm.	Indifferent	20	23	22	18	14	22	14	11	21	17	182
	Away	4	2	2	4	5	1	6	8	2	1	35
	Toward	1	0	1	3	6	2	5	6	2	7	33
Light on left side of worm.	Indifferent	16	22	20	19	17	23	17	13	13	17	177
	Away	4	1	4	4	3	2	6	7	6	1	38
	Toward	5	2	1	2	5	0	2	5	6	7	35

fifty-nine (71.8%) were directly forward, seventy-three (14.6%) were away from the light, and sixty-eight (13.6%) were toward it, leaving the almost insignificant amount of five (1%) movements to be ascribed to the directive influence of the light.

Summarizing these observations in a single table (Table V), it is

TABLE V.

Extent of exposure to light.	Whole worm. Per cent.	Anterior third. Per cent.	Middle third. Per cent.	Posterior third. Per cent.
Indifferent	65.6	54.6	67.2	71.8
Away	30.2	27.8	17.6	14.6
Toward	4.2	17.6	15.2	13.6
Light effect	26.0	10.2	2.4	1.0

apparent that the directive influence of light is much more effective on the anterior third of the worm than on the middle third, that it almost disappears in the region of the posterior third, and that no one of these thirds is as effective in influencing the direction of the animal's movements as is the whole of the body. In fact, the reactions obtained by illuminating the whole length of the worm are so much more considerable than any of those obtained from partial illumination, more even than the sum of these, that an error might be suspected in our method of work were it not well known that similar conditions exist in other organisms. For example, one can suffer any small portion of the surface of the hand to remain in water so hot that the whole hand cannot be held in it, thus demonstrating that the total amount of sensory surface exposed to stimulation has much to do with the quantitative character of the reaction.

The graded distribution of the sensitiveness of different parts of the worm to the directive influence of light as indicated in the foregoing experiments does not agree with the distribution of sensitiveness to *changes of light intensity* as worked out by Hesse ('96). According to Hesse the part of the worm most sensitive to changes in intensity is the anterior region, next in sensitiveness comes, not the middle, but the posterior region, and last of all the middle. Should Hesse's statements prove to be correct, they would afford in connection with the facts we have brought forward a conclusive argument in favor of the independence of photopathy, response to change in *intensity* of light, and of phototaxis, response to change in *direction* of light. But before this can be established, a quantitative study of the reactions of individual earthworms to both kinds of light stimulation must be successfully made.

Conclusions.

1. The earthworm Allolobophora fœtida (Sav.), when exposed to light of moderate intensity creeps away from the source of light, *i. e.*, is negatively phototactic.

2. The directive influence of a light of from 16 to 13.5 candle power at a distance of 50 cm. was measured by the percentage of head movements directed away from the light after deducting those made in the same direction but due to other stimuli than light. This percentage was 26 when the whole length of the worm was illuminated, 10.2 when only the anterior third was illuminated, 2.4 when the middle third was illuminated, and 1 when the posterior third was illuminated.

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THE PHYSIOLOGICAL ACTION OF THREE POISONOUS TOADSTOOLS — AMANITA MUSCARIA, AMANITA VERNA OR BULBOSA, AND AMANITA PHALLOIDES.[1]

By WILLIAM S. CARTER.

[*From the Physiological Laboratory, Medical Department of the University of Texas.*]

THE three poisonous mushrooms or toadstools which most frequently cause death in man have been studied in this investigation. These are the Amanita muscaria or "fly mushroom," the Amanita verna or Amanita bulbosa, and the Amanita phalloides or "death-cup." I have not investigated the toadstools containing minor or irritant poisons which set up gastro-intestinal symptoms that rarely cause death.

This report is based upon over ninety experiments, but is necessarily incomplete, as it has been impossible to procure a sufficient supply of the fungi in good condition for experimentation. Further, amongst animals of the same species there is a difference of susceptibility to these poisons, and therefore conclusions cannot be drawn from a small number of experiments. Most of the experiments were made with fungi dried at 40° C., or with glycerine or alcoholic extracts of the fresh growth.[2]

Amanita muscaria.

In animals the most prominent action of Amanita muscaria is upon the circulation. There is a very pronounced cardiac inhibition with a great fall of blood-pressure immediately after the intravenous injection. With large or rapidly given doses there may be complete inhibition of the heart for a variable time but the heart starts to beat again spontaneously and the pressure gradually returns. Once the heart stopped for one minute and fifty seconds, but at length resumed its contractions, and the normal blood-pressure was slowly regained.

[1] Being a report of work done for the committee of the American Physiological Society for the study of poisonous toadstools.

[2] Thanks are due to Capt. Charles McIlvaine for identifying and supplying the toadstools used in these experiments.

When the cardiac inhibition is very pronounced, cutting both vagi does not cause it to disappear. The intravenous injection of atropine sulphate removes it at once. Cutting both vagi before injecting the toadstool, does not lessen the cardiac inhibition. There was complete cardiac inhibition for thirty seconds in one experiment, although the vagi were cut at the start. Atropine (0.002 gram) caused the inhibition to disappear entirely and the blood-pressure returned to the normal. Subsequently twenty-one times as much of the toadstool as had been given at first had no effect upon the circulation although the animal died later from the effects of the poison.

From the above results we may conclude that muscarine acts upon the inhibitory mechanism within the heart.

Sometimes the pressure was reduced to one half of the normal before the inhibition of the heart began. In one experiment there was very little cardiac inhibition at any time although the pressure fell to one half of the normal. It repeatedly happened that each injection of the toadstool caused a transitory fall of pressure after atropine had been given and cardiac inhibition had been thereby prevented. These facts indicate that some vaso-dilatation is produced by Amanita muscaria. This contributes to the fall of pressure, but is less prominent than the cardiac inhibition.

When the circulation was seriously altered by the poison, the respirations were generally slow and shallow. Usually the respiration stopped before the heart when death occurred quickly. In one instance death was due primarily to cardiac inhibition and the respiratory movements continued after the heart had stopped beating.

Vomiting and purging occasionally took place late in the poisoning, but much less frequently than in poisoning by Amanita verna or bulbosa. Apparently gastro-intestinal symptoms are much less common in animals when the poison is given intravenously than in man when it is taken by the mouth. Vomiting, purging, intestinal colic, and burning thirst are described as among the initial symptoms of poisoning in man.

The secretion of saliva is greatly increased in animals as well as in man.

The pupils were always contracted in poisoning by this fungus, but after giving atropine to relieve the cardiac inhibition they were widely dilated.

Convulsions were never observed in any of the animals poisoned by Amanita muscaria. As convulsions did not appear in frogs, we

may conclude that Amanita muscaria does not contain any poison which excites the motor centres. On the other hand the spinal cord was greatly depressed. In a few cases of poisoning by this fungus in man convulsions are reported, but not so commonly as in poisoning by the Amanita bulbosa or by Amanita phalloides.

Usually coma appeared late in the poisoning, but in a few instances it began early and persisted until death.

In making these experiments with Amanita muscaria, sixteen dogs, fourteen cats, one rabbit and nine frogs were used. There seemed to be no difference in physiological action or in the susceptibility of the mammals. The lethal dose for frogs is relatively much greater than for the other animals. It must be noted, however, that with different individuals of the same species there is considerable variation in the susceptibility to these poisons. Thus one dog was killed in twelve minutes by 0.033 gram dried Amanita muscaria per kilo of body weight; in another dog 0.223 gram per kilo caused death only after twenty hours. These are the widest extremes but they show how uncertain is the method of measuring the toxicity of a poison by the lethal dose or toxic equivalent. The same variation is commonly noted by bacteriologists determining in the virulence of toxines.

Atropine and muscarine are diametrically opposed in their actions upon the pupil, the secretions, and the heart. In judging of the antidotal value of any remedy, we should bear in mind the variable power of resisting poisons which exists in different animals. It is far safer to compare averages than individual experiments. The average of six experiments made on cats and dogs in which no antidote was used, shows the lethal dose to be 0.103 gram dried Amanita muscaria per kilo of body weight. In three of these death was rapid (twelve minutes to three hours); in the other three death was slow (fifteen to forty-eight hours). In four experiments in which atropine was used as an antidote, the average fatal dose was 0.335 gram per kilo and in every case death was slow.

From these results there can be no doubt of the antidotal value of atropine in poisoning by the Amanita muscaria. It should be borne in mind, however, that *late death* occurs notwithstanding the early administration of atropine. It is true that it was necessary to give considerably more of the poison to cause late death when the early cardiac inhibition was prevented by atropine. The atropine may be given at the same time as the poison, or even before it, but the result is the same.

Late death does not seem to be due to the cardiac inhibition. In some cases the pulse and blood-pressure remained normal for a considerable time after the poison and yet death finally resulted. In one animal the pressure was taken again six hours after the poison had been given; the pressure was normal and the cardiac inhibition had almost disappeared, but the animal was still very ill.

These facts indicate that Amanita muscaria contains some poison or poisons to which atropine is not an antidote. These probably cause death late in the poisoning. This is an important fact in the treatment of poisoning by Amanita muscaria in man.

Both fresh and dried Amanita muscaria were extracted by distilled water and by 5 per cent solution of sodium chloride, but no difference could be detected in the action of the two solutions. Extracts of the fresh growth in alcohol (95 per cent) and in glycerine were also used. There was very little difference in the two, with one exception — when the alcoholic extract was used the cardiac inhibition was more pronounced; when the glycerine extract was used there was a greater fall of blood-pressure from the vaso-dilatation. In one case the pressure was reduced by the glycerine extract to one half of the normal with practically no inhibition of the heart. Possibly alcohol extracts relatively more of the muscarine, while glycerine extracts more of the other poisons.

Boiling did not lessen the toxicity of the fungi that had been dried at 40° C. With the fresh growth, our experiments on this point are incomplete.

Drying at 40° C. appeared not to diminish the toxicity. This statement is based upon a comparison of the lethal doses of dried fungi with those obtained with glycerine and alcholic extracts of the fresh growth. Unfortunately a solution of magnesium sulphate was used to prevent coagulation of the blood in recording the blood-pressure in the first experiments with fresh fungi and this prevents a direct comparison, as is explained elsewhere.

Amanita verna or Amanita bulbosa.

The most conspicuous effect of Amanita bulbosa is that upon the circulation. Inhibition of the heart occurred in about one-half of the experiments, but was not so pronounced or so persistent as with Amanita muscaria. Slight inhibition occurs when the vagi are cut before the poison is given, but is never so great as when they are

intact. Section of the vagi after cardiac inhibition has been produced by the poison does not entirely do away with inhibition. Atropine, however, causes the cardiac inhibition to disappear. It would seem, therefore, that Amanita bulbosa acts both upon the cardiac inhibitory centre in the medulla and upon the inhibitory mechanism within the heart.

In some experiments a great fall of blood-pressure occurred before the cardiac inhibition appeared, while in others, there was no inhibition of the heart. In this way the blood-pressure may be reduced to one third, or even to one-fifth of the normal. In some instances the pressure showed no inclination to return to the normal after the cardiac inhibition disappeared but remained low until death. After the administration of atropine, inhibition of the heart was removed, but the pressure remained low or returned very slowly. In poisoning by Amanita muscaria the pressure returned very rapidly when cardiac inhibition was stopped by atropine.

It is evident from this that the chief action of Amanita verna is upon the vaso-motor system, causing a widening of the blood-vessels. Amanita verna contains muscarine, or some body allied to it, which produces cardiac inhibition. The latter is only of secondary importance in bringing about the fall of blood-pressure.

Respirations were usually slow and frequently irregular. When death ensued rapidly the respirations always ceased before the heart stopped beating.

The secretion of saliva is somewhat increased. Retching and vomiting occurred twice, while purging occurred in one-fourth of the experiments in which the poison was given intravenously.

Convulsions were very frequently observed in poisoning by this fungus. Of fourteen dogs and cats receiving lethal doses two had convulsions. Of eight frogs, four had convulsions; two of the other four displayed great restlessness and increased spinal irritability. The convulsions were tetanic in nature. This fungus apparently contains some poison which stimulates the spinal cord. Coma appeared late in the poisoning, after the blood-pressure was greatly reduced.

No difference exists in the susceptibility of dogs and cats to the poisons of this fungus.

Atropine appeared to be of very little value as an antidote, although the experiments were not sufficiently numerous to warrant a positive conclusion. Atropine was generally given too late in the poisoning to be of any real value. In two experiments, however, it

was given early, but apparently without any effect. The lethal doses in these two experiments were lower than in some others in which no antidote was given. Considering the fact that the chief disturbance of the circulation in poisoning by Amanita verna is vasodilatation and not cardiac inhibition, it is not surprising that atropine is not so valuable as an antidote as in Amanita muscaria poisoning. Amanita verna is relatively three times as toxic as Amanita muscaria.

Distilled water and physiological salt solution were used for extracting fresh and dried Amanita verna, but no difference could be noticed in the action of the two extracts. An alcoholic extract seems to be less toxic than the glycerine extract. The average of two experiments with the alcoholic extract gives the lethal dose as 0.167 gram (fresh) per kilo; the average of three experiments with glycerine extracts gives the lethal dose as 0.068 gram per kilo.

In a single experiment boiling the fresh toadstools appeared to lessen the toxicity, but this cannot be stated definitely, as magnesium sulphate was used in the pressure-bottle at that time.

For the reason just mentioned we cannot compare the results obtained with fresh toadstools with those obtained after drying. The average lethal dose of the dried Amanita verna was 0.034 gram [1] per kilo, which would be equivalent to 0.217 gram in the fresh state. As this is very little above the lethal dose of alcoholic extracts (0.167 gram), it seems improbable that the toxicity would be diminished by drying.

Amanita phalloides.

The action of Amanita phalloides upon the circulation is very similar to that of Amanita verna or bulbosa.

There is some inhibition of the heart, but it does not occur constantly; neither is it so pronounced nor so lasting, as in poisoning by Amanita muscaria. Entire inhibition occurred only once, and section of the vagi caused it to completely disappear. The injection of Amanita phalloides after section of the vagi, does not cause much inhibition of the heart. This indicates that the chief action is upon the inhibitory centre in the medulla. It will be remembered

[1] Drying at 40° C. caused a loss of 84.4 per cent in the weight of the fungi. The weight in the fresh state is therefore equivalent to 6.4 times the weight after drying.

that the Amanita muscaria acts upon the inhibitory nerve cells in the heart. This difference may be merely one of degree, or it may be due to other substances or varying amounts of muscarin.

There was a great fall of blood-pressure independently of the cardiac inhibition. In some experiments the pressure fell as low as one half and even to one fifth of the normal without cardiac inhibition; in others it preceded the slowing of the heart, or came on after the inhibition had passed off. When the vagi were severed during the cardiac inhibition after giving the poison, the pressure either remained low or rose only momentarily and quickly fell again. During a convulsion the pressure rose, but fell as soon as the convulsion was over. Stimulation of the sciatic nerve failed to cause any rise of pressure although the heart was accelerated.

It is quite apparent that the vaso-dilatation is the chief cause of the fall of blood-pressure in poisoning by Amanita phalloides and that the vaso-dilatation is due to paralysis or paresis of the vaso-motor centres.

The respirations were slower than normal and at times irregular. When death was speedy the respiration stopped first.

In twenty-five dogs poisoned by the intravenous or subcutaneous injection of dried Amanita phalloides or extracts of the fresh fungus, vomiting occurred only twice and purging once. The secretion of saliva was not increased as much as by the other two toadstools.

The tendency to purgation does not seem to be as great as in poisoning by Amanita verna.

Usually coma came on late in the poisoning, but when the poison was given rapidly, or in large quantities, coma came on early and persisted until death.

Mild tetanic convulsions occurred twice in twenty-five dogs; twelve frogs were poisoned with different preparations (dried fungi, and alcoholic and glycerine extracts of the fresh fungi), but convulsions did not appear in a single instance. In two rabbits poisoned with fresh Amanita phalloides, given intravenously, convulsions came on during the rapid fall of blood-pressure, but as magnesium sulphate was then used to prevent coagulation of the blood these results are open to doubt.

From the failure to produce convulsions in frogs, it would appear that this fungus does not excite the spinal cells as does Amanita bulbosa.

Some mycologists regard the Amanita verna or bulbosa as only

a variety of the Amanita phalloides and not as a distinct species.[1] It seems difficult to distinguish between this fungus and colorless forms of Amanita phalloides by the structure alone. This difficulty of identification may explain why convulsions occur in men poisoned by Amanita phalloides so much more frequently than they were observed to occur in animals. There may, of course, be some variation in the poisons contained in different growths.

Our experiments with Amanita bulbosa were not very numerous, as only a limited amount of the toadstool could be obtained, but from the greater prominence of the nervous symptoms, they indicate that the Amanita bulbosa is distinct from the Amanita phalloides.

Almost all the experiments with Amanita phalloides were performed upon dogs, and no comparison can be made with the action upon other mammals.

Atropine is of little service in poisoning by Amanita phalloides, except that it causes the inhibition of the heart to disappear. As this condition is not so serious as the vaso-dilatation in poisoning by this fungus, atropine has very little practical value.

The average of seven experiments on dogs in which dried Amanita phalloides was given without any antidote shows the lethal dose to be 0.128 gram per kilo. In seven experiments in which atropine sulphate was given the average lethal dose was 0.172 gram (dried) per kilo.

The average of four experiments in which recovery occurred without any antidote gave an average dose of 0.096 gram per kilo. These experiments were made to determine the minimal lethal dose.

Transfusion of physiological salt solution was tried late in the poisoning in three experiments. The injections were made directly into the vein and the pressure improved after them, but all the experiments ended fatally and the lethal doses were no greater than in other experiments where no effort was made to combat the poison.

Suprarenal extract from its direct action upon the walls of the blood-vessel would, perhaps, be more strongly indicated in this poisoning than those drugs which cause vaso-constriction by acting upon the vaso-motor centre. The pressure is increased, for a time at least, by injecting large amounts of physiological salt solution either intravenously or hypodermatically. This has the additional advantage of increasing the renal secretion and thereby aiding in eliminat-

[1] McIlvaine: American fungi, p. 10.

ing the poison. The suppression of urine seen in man is probably due to the low blood-pressure. It seems a rational procedure, and should be tried in conjunction with other remedies.

Boiling solutions of dried Amanita phalloides did not alter the toxicity.

Extracts of the fresh growth in alcohol and in glycerine showed no difference in their actions.

Dried Amanita phalloides was extracted in 5 per cent NaCl and in distilled water but there was no difference in the action of the two solutions.

The average lethal dose of glycerine and alcoholic extracts was 1.110 grams (fresh) per kilo. This is equivalent to 0.173 gram of the dried. The average lethal dose of the dried (seven experiments) was 0.128 gram per kilo.

From this it appears that drying does not lessen the toxicity.

In a single experiment an effort was made to determine whether or not there is a volatile poison in the Amanita phalloides. A one per cent extract of the fresh growth was distilled until three-fourths of the original fluid had passed over. This distillate was then injected into a dog with no effect. Dr. J. P. Arnold kindly repeated this experiment for me, injecting large quantities of the distillate into frogs and rabbits with no effect. We may conclude that Amanita phalloides does not contain a volatile poison.

The toxicity of the Amanita phalloides appears to be about the same as that of the Amanita muscaria, while Amanita verna or bulbosa, is approximately three times as toxic.

The average doses of the dried fungi required to kill when no antidote was given were as follows: —

Amanita muscaria (six experiments) 0.103 gram (dried) per kilo of body weight; for Amanita phalloides (seven experiments), 0.128 gram per kilo; with Amanita bulbosa or verna, 0.034 gram per kilo.

Toadstool poisoning differs from most forms of poisoning in the length of time which, in fatal cases, elapses before death. In man, death usually comes on two or three days after the ingestion of the poison and may be delayed as long as a week. In animals, the time varies from a few hours to several days. During our first experiments it seemed impossible to kill dogs with Amanita muscaria. At that time only the *early* effects were essayed. When death did not come on at the end of two or three hours the animal was

killed by chloroform. It was soon found however, that these animals might recover from the early disturbance of the circulation, either spontaneously or with the help of atropine, and yet they died from the *late* effect after the wound was closed and the animal returned to the kennel. This may occur forty-eight hours or more after the intravenous injection of the poison. The contrast between the early and late effects is more marked with Amanita muscaria than with Amanita verna or Amanita phalloides. With these latter two toadstools, the late effect appears to be merely a continuation of the early symptoms and the circulation is not restored to the normal.

In all cases, however, death comes on later than in poisoning by alkaloids or by inorganic poisons unless the latter bring about tissue changes (*e. g.*, phosphorus).

Husemann believed death to be due to fatty degeneration in the organs of the body. Tissues taken from dogs and cats dying from the late effects of Amanita muscaria and Amanita phalloides were fixed in 2 per cent formalin and stained by hematoxylon and were found normal. Death is therefore not due to extensive degeneration of the heart, kidney, liver, and other tissues.

Mr. V. K. Chestnut in a bulletin published by the United States Department of Agriculture (Circular No. 13, p. 23) states that death in Amanita phalloides poisoning is caused by a destruction of red blood corpuscles. The authority for this statement is not given. The conclusion has probably been based upon the extreme venosity of the blood seen late in the poisoning from the disturbances of the circulation and respiration.

The blood corpuscles have been counted repeatedly in poisoning by all three of the Amanitæ studied and no material reduction occurred in any of the experiments. It can be stated positively that death is not due to destruction of the red blood corpuscles. Further, the blood was examined with the spectroscope to see if any hæmoglobin compound had been formed. In every case the absorption bands of oxy-hæmoglobin were observed as usual. No stable compound is formed with the hæmoglobin. As many of the blood examinations were made a very short time before the death of the animal, they leave no doubt as to the hæmoglobin or the corpuscles.

It is well known that the toxin of diphtheria does not cause death in a short time unless given in overwhelming quantities. The action of this poison appears to be somewhat similar to that of the poisons of the three toadstools studied in this paper. The diphtheria toxine

I.—AMANITA MUSCARIA. FROG.

No. of exp.	Weight of frog. Grams.	Strength of solution used. %	Material used for extracting toadstool.	Mgm. of poison per gram of body weight.	Result.	Time.	Respiration.	Inhibition of heart.	Remarks.
1	40	5	20% ext. in 50% alcohol.	0.000375	No effect				
2	25	10	50% alcohol	0.006	Recovery	3 hrs.	Feeble and irregular	Very marked	Greatly depressed. Feeble response to stim. of skin. Some twitching. Sprawled out; could not turn over when placed on back. Better after 1½ hours and able to get over.
3	30	10	50% alcohol	0.010	Recovery	4 hrs.	Depress'd. Stopped for a time	Marked	Great depression; very feeble response to mechan. stim. of skin. Some twitchings in legs. Sprawled out. Does not draw legs up or make effort to turn over when placed on back.
4	28	10	50% alcohol	0.0143	Late death	Observ'd 6 hours. Died in night.	Paralyzed	Pronounced. Heart feeble late.	Nervous symptoms same as in above experiments but did not recover from them.
5	40	2 (dried)	H_2O	0.001	Recovery	5 hrs.	Slow	Distinct	Sprawled out but gets over when placed on back and draws legs up when they are extended. Nervous symptoms improved after 2 hours.
6	45	2 (dried)	H_2O	0.00177	Recovery	2½ hrs.	Slow and feeble	Heart slow and strong	Sprawled out; draws up legs when they are extended; cannot get over when placed on back. Symptoms greatly improved after 2 hours.

7	52	2 (dried)	H_2O	0.001923	Death	5¼ hrs.	Paralyzed 1¾ hours after inj.	Distinct, early. Heart did not respond to stim. 5 hours after inj.	Sprawled out and could not get over or respond to stimulation.
8	25	2 (dried)	5% NaCl	0.0016	Death	2 hrs.	Paralyzed	Could not see heart beat 1 hour after injection	Responds feebly to strong mechan. stim. of skin but cannot get over when placed on back. Sprawled out.
9	33	2 (dried)	5% NaCl	0.0018	Death	3–4 hrs.	Irregular and feeble	Distinct, early; late beats weak and slow. Finally no response to pressure over heart	Very weak and sprawled out during 1st hour after injection. Still able to turn over when placed on back 2 hours after inj. 2½ hours after inj. can only see heart beat by pressure over heart.

produces profound structural alterations of the neurons of the nervous system as well as degeneration of the glands and muscle. Pieces of the brain and spinal cord were stained by silver impregnation (silver phosphomolybdate) in fatal cases of poisoning by Amanita phalloides and Amanita muscaria. There were none of the structural changes of the neurons described in certain acute toxæmias — such as loss of gemmulæ, varicosity of the dendrites, etc.

The cause of this late death remains unexplained.

The protocols of several experiments are appended.

II.—AMANITA BULBOSA. FROG.

No. of exp.	Weight of frog. Gms.	Strength of solution used. %	Material used for extracting toadstool.	Mgm. of poison per gram of body weight.	Result.	Time.	Respiration.	Inhibition of heart.	Remarks.
1	25	3 (fresh)	20% ext. in pure glycerine	0.0036	Recovery	1 day+			Convulsion 20 min. after injection; continued 2 hrs. and then gradually disappeared. Frog ill next day and very weak, but recovered.
2	25	3	20% ext. in 50% alcohol	0.0036	Death	Dead next day	Slow	Slow and regular (inhibition)	Frog ill in ½ hr. and remained so 2¼ hrs. Sprawled out but able to turn over. Found dead next morning.
3	30	3	Glycerine	0.003	Death	Dead next morning	(Not noted)	(Not noted)	
4	20	3	Glycerine	0.0045	Death	Dead next day	(Not noted)	(Not noted)	Convulsions began 20 min. after injection and continued for over 2 hrs. Much less severe after 2 hrs. Exp. late in afternoon. Found dead next morning.
5	34	5	Glycerine	0.0029	Recovery	Very ill for 2 hrs.	Accelerated early		Convulsion 10 min. after injection; continued 1½ hrs. Very ill; sprawled out.
6	39	5	Glycerine	0.0038	Recovery	Very ill			Convulsions began 13 min. after injection and continued 1¾ hrs. Very ill, but not as severe symptoms as others.
7	26	5	50 % alcohol	0.0038	Recovery	Sick 2 hours	Accelerated early		Great restlessness began 20 min. after injection and continued over an hour. Quiet after 2 hrs.
8	33	5	50 % alcohol	0.0045	Recovery		Accelerated		Restlessness 40 min. after injection, only continued 20 min. longer. In struggling, frog tore ligature from skin and some fluid may have escaped from lymph sac.

The wound in the skin through which the poison was injected into the dorsal lymph sac was ligated in experiments 5, 6, 7, and 8, but was left open in experiments, 1, 2, 3, and 4.

No. of exp.	Weight of frog. Gms.	Strength of solution used. %	Material used for extracting toadstool.	Mgm. of poison per gram of body weight.	Result.	Time.	Respiration.	Inhibition of heart.	Remarks.
1	25	3	20% ext. in glycerine	0.0012	No effect				Some fluid may have escaped from the lymph sac.
2	28	3	Glycerine	0.0021	Recovery			Distinct	Frog sick, but always able to turn over when placed on back.
3	30	3	Glycerine	0.003	Death	During night		Distinct	
4	30	3	95% alcohol	0.003	Recovery			Distinct	Very ill ½ hour after inj., but recovered.
5[1]	30	3	Glycerine	0.003	Death	(Next day)			Very ill for 2 hours after injection, and died next day.
6	23	10	95% alcohol	0.00217	Recovery		Accelerated		
7	20	10	Alcohol extract	0.005	Death	½ hour			Appeared to be dead ¼ hour after injection but heart continued to beat for ½ hour.
8	28	10	Alcohol extract	0.007	Death	5½ hrs.			Sprawled out 20 min. after inj. No response to stim. of skin. Remained in this condition till heart stopped (5½ hours).
9	30	10	Alcohol extract	0.010	Death	1 hour	Paralyzed 15 mins. after inj.	Pronounced ½ hr. after inj. Ceased beating 1 hour after injection	Sprawled out and no response to mechanical stimulation of skin.

The wound in the skin through which the poison was injected into the dorsal lymph sac was ligated in experiments 6, 7, 8, and 9, but was left open in the other experiments; in the latter some of the poison may have escaped.

[1] Frog used in experiment 4.

IV.—EXTRACTS OF FRESH AMANITA BULBOSA OR VERNA. DOG.

No. Exp.	Weight of dog. Kilos.	Strength of sol. used.	Material used for extracting toadstool.	Gm. of poison per kilo of body weight.	Result.	Time.	Antidote.	Respiration.	Gastro-intestinal.	Nervous symptoms.	Cardiac inhibition.	Blood-pressure.	Remarks.
25	10.0	2	Pure glycerine (20% ext.)	0.06	Early death.	20 min.	Atropine early	Irregular	Vomited	None	Distinct. Came on with fall of pressure. Disappeared after atropine. Came on again late and did not disappear after cutting vagi	Gradual fall after first injection. Atropine removed inhibition but did not restore the pressure. Pressure remained one-half of normal	Bloody fluid from nose late in poisoning.
26	8.52	2	95% alcohol (20% ext.)	0.188	Recovery from early effect. Killed by chloroform	20 min.	..	..			None	None	Had practically no effect. Late effects not tried for.
27	5.91	2	Glycerine	0.119	Death	30 min.	Atropine late	Slow			Vagi cut at start. Marked inhibition; removed by atropine	Pressure did not fall much until late	Inhibition with vagi severed is quite as great as when they are intact.
28	8.07	2	Glycerine	0.025	Death	1 hour 5 min.	Atropine late	..			Vagi cut at start. No inhibition	Pressure reduced to one-third of normal without any inhibition	Corpuscles counted three times during poisoning; no reduction in erythrocytes.
33	15.23	3	50% alcohol (20% ext.)	0.147[1]	(See protocol.) Death not due to this alone	3 hrs.	None	..			Practically none	Pressure already low from dried Am. bulb. before alcohol ext. given	

[1] Other poison (dried Am. bulb.) given before alcoholic ext.

V. — EXTRACTS OF AMANITA PHALLOIDES MADE FROM FRESH FUNGI. DOG.

No. of exp.	Weight of dog. Kilos.	Strength of sol. used. %	Material used for extracting toadstool.	Gms. of poison per kilo of body weight.	Result.	Time.	Antidote.	Respiration.	Gastro-intestinal.	Nervous symptoms.	Cardiac inhibition.	Blood-pressure.	Remarks.
19 B	5.97	1	95% alcohol	0.151 (fresh)[1]	Early death	¾ hour	Atropine very late; no effect	Not noted	Retching	Genr'l c'nvulsions; coma late	Distinct early; disappeared entirely after section of vagi	Pressure fell to one-third after all inhibition had disappeared	Dog nearly dead; killed by chloroform.
20	4.37	1	Glycerine and 95% alcohol	1.36	Early death	1⅓ hrs.	None	Not noted	None	None	Complete twice just after injections. Disappeared largely after cutting vagi	Pressure fell out of all proportion to inhibition toward end of the experiment	Pressure did not fall much except with complete cardiac inhibition until after last injection.
26 A	8.52	2	Glycerine and alcohol	0.797	Recovery from immediate effect	1¼ hrs.	None	Not noted			Large pulsations in tracing. Frequency reduced to three-fourths of normal	Pressure fell after each injection, but recovered promptly	As pressure recovered entirely animal was used for Am. bulbosa.
30 B	18.07	2	Glycerine and 95% alcohol	0.153[2]	Killed by chloroform	25 min.	None				Not marked or serious	Great fall of pressure, out of proportion to inhibition of heart	

[1] After 0.159 gram dried per kilo had been given. See Remarks.

[2] After having given 0.088 gram dried.

VI.—DRIED AMANITA VERNA OR BULBOSA. DOG AND CAT.

No. of exp.	Animal. Weight. Kilos.	Strength of sol. used. %	Material used for extracting toadstool.	Gm. of poison per kilo of body weight.	Result.	Time.	Antidote.	Respiration.	Gastro-intestinal.	Nervous symptoms.	Cardiac inhibition.	Blood-pressure.	Remarks.
16	9.32 Dog	0.076 gms. in 100 c.c.; equal to 0.5% sol. of fresh	H_2O + 0.6% NaCl	0.064	No effect	20 min.							
17	4.09 Dog	0.1	0.6% NaCl	0.0193	Death	1 hour	None	Irregular	Retching	None	Very pronounced; partially relieved by cutting vagi	Pressure fell before inhibition came on. Pressure rose slightly after section of vagi	Animal almost dead; killed by chloroform.
33	15.23 Dog	1 and 1.5	H_2O	0.032 or 0.049	Death	3 hours					Some, but not serious	Pressure fell to one-fifth of normal without inhibition	Attempt to see if poison is diffusible through membrane.
44	2.39 Cat	0.2	H_2O	0.15	Death	$1\frac{3}{4}$ hrs.		Accel.	Purging twice	Coma	None	Pressure reduced to less than half without any inhibition of heart	
45	4.43 Dog	0.2	H_2O	0.069	Death	2 hours			Purging	Coma	Distinct but transitory	Pressure reduced very little. Wound closed up and late effect tried for	Blood examined spectroscopically, but no change found.

THE FUNCTION OF THE BRAIN IN PLANARIA MACULATA.

By CHARLES RUSSELL BARDEEN.

[*From the Department of Anatomy in the Johns Hopkins University, Baltimore.*]

IN a recent article[1] I described several characteristics of the functional activity of the nervous system in Planaria maculata. I therein called attention to a fact previously noted by Loeb,[2] that the body of a Planarian from which the head, including eyes and brain, has been removed, is still capable of "spontaneous" movement, and of reacting definitely towards light. Loeb showed that the headless body of a planarian reacts more slowly towards light and other stimuli than does that of a normal worm. From this and similar experiments conducted on other invertebrates Loeb concludes that the function of the central nervous system is to act as a quick, convenient, but not always necessary path for the distribution of sensory impulses to motor organs.[3]

In my previous article I showed that even a small piece of a Planaria maculata may exhibit spontaneous movements, react to light, and give rise to specific internal regenerative changes, provided that it contain a part of at least one of the two nerve cords; otherwise no such activities take place. In headless pieces the portion of nerve cord left in each piece acts as a central nervous system until a new nervous system has been regenerated. In this animal sensory-motor coördination takes place only through a central neural apparatus.

Many of the reactions of a Planaria maculata are exhibited when the brain is removed. Are we, therefore, to conclude that the most complicated part of the central nervous system of this worm has no specific functions?

On *a priori* grounds one might assume that the function of the brain in these creatures, as in most animals, is to act as a centre of association for the control of the more complex relations of the indi-

[1] BARDEEN: This journal, 1891, v, p. 1.

[2] LOEB: Archiv für die gesammte Physiologie, 1894, lvi, p. 247.

[3] LOEB: Comparative physiology of the brain and comparative psychology, 1900, pp. 38, 45, 86, 101, 125.

vidual to its environment. The most complex activities of the planarian which I could well study in isolated individuals were those having to do with feeding. If a planarian be removed from its natural habitat and be kept in pure water for two weeks it will usually be rendered "hungry." If such a planarian be resting on the side of a dish it may not be attracted for some time by a bit of snail placed in the water near it. But if the dish containing the planarian be brought from the dark into the light the resting planarian will commonly be aroused into activity. Once in motion it quickly finds the food material. When the swimming or crawling animal comes within a centimetre or two of a bit of snail it is apt to turn its head in the direction of this food and to proceed toward it. It is difficult to determine the source of the impulse which gives rise to this purposeful activity. It is possible that the auricular appendages here act as delicate organs capable of stimulation by slight currents in the water set up by the minute organisms that prey at once upon the flesh of the dead snail. At any rate, after the first planarian has found the piece of food others are more quickly brought to the spot.

The planarian gently approaches the piece of food. Having reached this the animal raises its head and sways it to and fro as if to examine the food. The auricular appendages meanwhile are raised and exhibit a slight wave-like motion. As a rule, the planarian now crawls upon the piece of snail and finally wraps its body about a small bit of its flesh. The pharynx is extended and becomes attached at its distal extremity to some of the food stuff. The worm now often turns about and faces away from the piece of snail so that the pharynx is extended at full length and in a direct line with the axial gut. It seems probable that at the mouth of the pharynx secretions are furnished which have a powerful digestive action when applied to animal tissue. Strong planarians often prey upon weak ones. In such instances the strong individual attaches its pharynx somewhere upon the body of the weak one, usually near the head. The flesh of the victim seems to melt as it disappears with great rapidity into the pharynx. Along the margin of the wound the flesh turns black. Such scarred pieces are frequently seen where planarians are found. In a similar way the tissue of the snail seems to dissolve partially in the pharyngeal secretions, and it is then rapidly poured into the axial gut and thence into all of the intestinal branches.

Occasionally a planarian stops before reaching a bit of food and extends the pharynx toward it. If this be reached feeding will take place as before.

In this process of natural feeding we have therefore to distinguish two processes, finding and recognizing the food, and devouring it. Both sets of processes require a coördinated complex of movements upon the part of the worm. The following experiment was performed to determine whether a brain is necessary for the performance of these processes.

Experiment I. — Feeding of Decapitated Worms. From some planarians found in a small stream near Baltimore I selected thirty well developed individuals. These I placed in tap water in a clean glass dish and kept them for two weeks without food. I then divided them into three lots of ten each and treated them as follows. In the first lot the heads were severed just posterior to the auricular appendages (e–f, Fig. 1). In the second the tail was severed just posterior to the pharyngeal pocket (g–h, Fig. 1); by this procedure more tissue was removed from each body than by the removal of the heads in Lot 1 and as much tissue was exposed at the wound. The third lot was left uninjured for purposes of control.

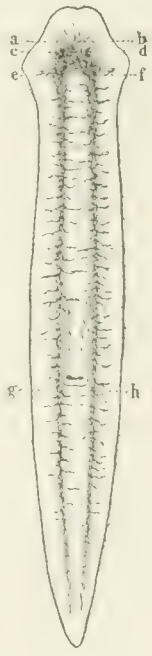


FIGURE 1.

Each lot of worms was placed in a separate dish and the dishes were left in a dark place for twenty-four hours. They were then brought into the light. The transition from darkness to light set the worms in all three dishes in locomotion. In each dish a small portion of snail was placed. Within ten minutes all of the normal worms had found the morsel of snail and were eagerly devouring it. Within the same length of time all of the tailless pieces, except one, were attached closely to the portion of snail in their dish. On examination it was found that in the unattached worm the tip of the pharynx had been removed in the attempt to cut off the tail. At the end of half an hour not one of the headless pieces of Lot 1 had attacked the portion of snail placed in the same dish. I then spent fifteen minutes in placing portions of snail in the way of the headless pieces and at last succeeded in getting a headless piece to eat. This piece happened to settle down upon a bit of food which the pharynx reflexly engulfed. Several times other pieces settled down for a moment on a morsel of food and the pharynx was reflexly extended. But the worm moved on without responding to this opportunity to rest while food was consumed. In other words these worms failed to find food and failed usually to react normally when in contact with it.

From this experiment it seems fair to conclude that the head is an organ necessary for putting the planarian into definite relations with its natural food. The headless pieces were experimented with from day to day until the heads had regenerated. Only after the heads were well formed did these pieces behave in a natural manner toward food placed in the dish. However, if left constantly near food, headless pieces may at times consume that with which they accidentally come in contact.

On the other hand, the simple reflexes of extending the pharynx and of swallowing are preserved after removal of the head. I found, by repeated trials, that one of the headless pieces could usually be made to eat if it was placed on its back on a slide in a small drop of water. Under the conditions mentioned the pharynx is usually protruded and will engulf bits of food placed in the mouth.

That the loss of power to recognize food exhibited by the headless worm is not due to the pathological action of the wound as such is shown by the normal reactions of the tailless pieces.

Experiments similar to that described above were repeated with like results. The normal reactions of these worms are best seen in specimens freshly collected. As Loeb pointed out,[1] the power to respond to stimuli becomes weakened in worms long in confinement.

Observations were now made to determine how much of the planarian brain is necessary for the recognition of food.

Experiment II. — Feeding of Worms from which a Part of the Head was Removed. Twelve fasting worms were divided into two lots which were treated as follows. (1) From six worms I separated the end of the "nose" by a section midway between the eyes and the anterior extremity of the body a-b, Fig. 1. (2) From six worms the anterior portion of the head was separated by a section through the region of the eyes, c-d, Fig. 1. In each dish were placed several portions of snail. All the planarians in Lot 1 ate of the food within fifteen minutes. In Lot 2 only one took any notice of the food. This one after half an hour became attached to a portion of snail. On removal and careful examination it was found that in this piece the section had passed in front of the eyes instead of through them.

From this experiment it appears that complex reactions, such as the recognition of food, cannot be performed by planaria unless the brain is intact as far forward as the anterior margin of the eyes.

[1] *Op. cit.*

Conclusions.

1. In Planaria maculata nerve cords and brain constitute the central nervous system.

2. A fragment of this planarian will exhibit no sensory-motor coordination unless it contain a part of the central nervous system.

3. If the anterior extremity of the body be removed by a transverse section passing through the eyes or through the body posterior to this region the worm will lose the power of recognizing food and of reacting normally towards it. The simple swallowing reflexes are maintained.

4. For the more complex reactions of the individual the brain must be intact as far forward as the anterior margin of the eyes.

A TYPE OF REACTION BY WHICH SODIUM CARBONATE AND HYDROCHLORIC ACID MAY BE FORMED IN THE ANIMAL ORGANISM.

By THOMAS B. OSBORNE.

[*From the Research Laboratory of the Connecticut Agricultural Experiment Station.*]

SOME time ago, in a paper on Some Definite Compounds of Protein Bodies,[1] I called attention to the basic properties of protein substances and showed that preparations of the crystalline globulin edestin, as usually obtained from the hemp-seed, are mixtures of salts, chiefly hydrochlorates and sulphates. The nature of this combined acid depends upon the salts present in the solution at the time of precipitation, the acid of the seed sufficing to enable some of each of the acids of these salts to combine with the protein.

These facts led me to examine the precipitate produced by carbonic acid, in a dilute sodium chloride solution of edestin, as it seemed possible that this might consist chiefly of hydrochlorate.

A quantity of a relatively pure preparation of edestin, which had been several times recrystallized from a warm dilute sodium chloride solution by cooling, was suspended in water and made exactly neutral to phenol phthalein by decinormal potassium hydrate solution. The edestin thus neutralized was washed with water and dissolved in sodium chloride brine. The solution was diluted with water until it became slightly turbid and carbonic acid gas was passed through it until the edestin appeared to be completely precipitated. This was filtered out, washed thoroughly with one per cent sodium chloride solution and then with fifty per cent alcohol, until no chlorine could be detected in the washings, dehydrated with absolute alcohol and dried over sulphuric acid. The substance thus prepared, while insoluble in dilute sodium chloride solution, was largely soluble in pure water, as well as in strong sodium chloride brine, yielding solutions acid to litmus and to phenol phthalein. To neutralize one gram to the latter indicator 1.0 c.c. of decinormal potassium hydrate solution was required. Fifteen grams of this preparation were treated with

[1] OSBORNE: Journal of the American Chemical Society, 1899, xxi, p. 486.

freshly boiled water and 28.5 c.c. of decinormal potassium hydrate solution, diluted with much water, were added. The edestin, which separated completely from the solution, was then filtered out, washed with water and filtrate and washings evaporated on a water-bath. The residue was dried at 110° and analyzed with the following results:

	Gram.
Organic matter	0.0222
Inorganic matter . . .	0.2123
Total residue . . .	0.2345

The inorganic residue contained:

	Gram.
Potassium chloride . .	0.1994
Potassium sulphate . .	0.0153

The potassium added was equivalent to 0.2127 gram of potassium chloride, so that over 93 per cent of the potassium added was recovered as chloride. From this analysis we find that with the 15 grams of edestin, equal to 13.5 grams dried at 110°, there had precipitated 0.0976 gram of hydrochloric acid or 0.072 per cent of the protein. Corresponding to this quantity of hydrochloric acid, 0.1417 gram of sodium carbonate must have been produced in the salt solution by the carbonic acid. It seems probable that by a similar reaction[1] both sodium carbonate and hydrochloric acid may be formed from sodium chloride in the organism, since there is always sodium chloride and protein matter present where carbonic acid is produced in the tissues.

[1] Cf. SCHULZ: Archiv für die gesammte Physiologie, 1882, xxvii, p. 454.

ON THE EFFECTS OF COMPLETE REMOVAL OF THE SUPRARENAL GLANDS.

By BENJAMIN MOORE and C. O. PURINTON.

[*From the Physiological Laboratory of the Yale Medical School*, 1900.]

IN a recent issue of this Journal we published a preliminary account of certain experiments on the effects of the entire removal of the suprarenal bodies in the cat.[1] We have since completed that series of experiments, and have added four experiments of a like nature performed upon goats.

Our previous communication referred chiefly to extensive ante-mortem clotting which was found in three cases in the heart and great vessels. Further experimentation has shown that ante-mortem clotting is not an invariable result of suprarenal removal, but that death may occur so far as is observable solely from the absence of the suprarenals. It is noteworthy that in each of the three cases in which ante-mortem clotting was observed, death ensued fairly rapidly after removal of the second gland, the longest interval observed being thirty-three hours. There was no sepsis in any of the three cases, nor clotting in the *vena cava* near the seat of the operation, and hence we are inclined to believe that the thrombosis was due to absence of the suprarenal and probably arose secondarily to the condition of the circulatory system brought about by absence of the suprarenal secretion. In the other animals, in which thrombosis did not occur, we obtained in some cases a longer survival and hence a better clinical picture of the condition resulting from complete suprarenal removal.

Experiments upon Cats.

We operated upon fifteen cats, seven of which survived sufficiently long to recover from the anæsthetic and the immediate surgical shock of the second operation, and we think that death was not due, in any of the seven cases, to surgical shock or sepsis, or in fact to any other cause than absence of the suprarenals. The animal in each case recovered completely from the intoxication of the anæsthetic, moved about the room, purred upon being stroked, and in most cases drank

[1] Moore and Purinton: This journal, 1900, iv, p. 51.

milk. The intervals between the removal of the first and the second gland varied in the different cases from twenty to thirty-nine days, and so far as could be observed, complete recovery had in each case taken place before the second operation was performed.

In our four latest experiments the animals were observed continuously day and night after the second operation, and the temperature, respiration and other symptoms were noted at short intervals.

The periods of survival were as follows: — three under twenty-four hours[1]; one for twenty-four hours; one for thirty-three hours; one for two days and four hours; one for four days and twenty-two hours.

In all cases careful *post-mortem* examinations were made either immediately or within a few hours of death. In no case was general peritonitis[2] or any septic condition found; nor was there any post-operative hæmorrhage or other discoverable cause of death except absence of the suprarenal glands; unless the cardiac thrombosis in three of the cases be regarded as such, and this we consider also as secondary to suprarenal deficiency. It was in every case found that the suprarenals had been completely removed,[3] and the side first operated upon was in each case completely healed. In no case could we find any accessory glands in the suprarenal region. No hypertrophy of the pituitary gland could be observed. The heart muscle was generally found abnormally pale, and mottled grayish patches were usually to be seen in the auricles.

The urine of the animals and also extracts of their various tissues were examined for the suprarenal chromogen, and in two experiments these extracts were also tested physiologically for the presence of the active suprarenal substance, but with negative results. This shows that autotoxication does not occur with these substances after suprarenal removal and hence confirms the view that they are secreted by the gland.

The evidence which we had obtained as to the hypertrophy of the

[1] These were the first three animals in which the second operation was accomplished. The operation was completed in each case between 5 and 6 P.M. and the animals were observed till about 10 P.M., when they had recovered from the anæsthetic and moved about and purred. These animals were not watched through the night and in each case were found dead on the following morning.

[2] Peritonitis was further excluded by clinical examination. The animals never assumed the drawn-up position, and there was no general tenderness of the abdomen to pressure.

[3] This was merely used as a control upon careful examination of the glands themselves made at the time of their removal.

remaining gland has been confirmed by the later experiments, as is shown in the following table: —

Weight of 1st gland removed. Gram.	Weight of 2d gland removed. Gram.	Interval between operations.
Right 0.280	Left 0.402	20 days.
Left 0.150	Right 0.281	39 days.
Right 0.196	Left 0.261	30 days.
Left 0.230	Right 0.322	27 days.
Right 0.205	Left 0.289	29 days.
Right 0.148	Left 0.151	

The following are the chief symptoms observable after the removal of the second gland: —

1. At first the animals appear quite normal after the operation. They move about the room and are able to drink. The thermometer usually shows a certain amount of pyrexia for a few hours, but there is no disturbance of respiration.

2. The animals later develop muscular asthenia; they do not move about unless disturbed and then they soon lie down again. This condition increases as the time advances.

3. The respiratory rhythm usually becomes markedly increased,[1] in some cases to more than treble the normal rate. The respiration becomes irregular, and in the end death takes place from respiratory failure, the heart continuing to beat for from one to four minutes after the respirations have ceased.

4. From one to three hours before death muscular twitchings appear all over the body. They are somewhat rhythmical, as many as ninety per minute having been observed. The twitchings can be called forth reflexly by touching the skin when they are less frequent. At times they seem to be connected with the inspiratory phase, although they do not occur during every inspiration when they first appear. The spasms are at first clonic but afterwards they tend to become tetanic, probably from increased frequency, and the animal usually dies in a tetanic convulsion. The clonic contractions appear

[1] In one animal, however, no increase in rhythm was observed.

first in the fore limbs or head and neck muscles, then in the abdominal muscles, and finally all over the body. Occasionally a twitch of the fore limbs takes place, followed an instant later by a similar twitch of the hind limbs, and at times an isolated twitch may be observed in one limb only, in the lumbar muscles, or in the lower jaw. It is interesting to note that during this period of clonic twitching, the muscular tonicity, as shown by a very exaggerated knee-jerk, is markedly increased, and this continues until death and for a few minutes thereafter. The slightest touch at knee or ankle causes a powerful contraction or series of contractions. The knee-jerk lasts from five to ten minutes after respiration has ceased.

The following extracts from the protocol of one of our experiments may serve to illustrate these points: —

Experiment, March 10. — Left gland removed; weight of gland, 0.15 gm.; weight of cat, 3 kg.

Experiment, April 18. — Right gland removed; weight of gland, 0.28 gm.; weight of cat, 3.1 kg. Operation completed at 3.30 P. M.; at 11 P. M., animal recovered from anæsthetic; T. 38.2°, R. 22.

Experiment, April 19. — At 1 A. M., animal awake; T. 39.4°, R. 23. At 3 A. M., animal asleep; on being roused it purrs and stands up; T. 39 3°, R. 22. At 5 A. M., T. 39.7°, R. 24. At 3 P. M. the temperature had reached 40.4°, R. 23; at this time the animal drank 100 c.c. of milk. At 8.40 P. M. the temperature reached its highest point, 41.3°, and then slowly sank during the night to slightly below the normal (38° to 39° in the cat).

Experiment, April 20. — At 11 A. M., T. 37.7°, R. 25, the animal took about 70 c.c. of milk. Up to this point nothing abnormal in the appearance of the animal had developed, but from this time onward the respiratory rate began to increase. At 4.15 P. M., T. 38.4°, R. 43; respirations strong and blowing in character; animal very listless, and lies quiet unless disturbed. At 6.43 P. M., strong jerking clonic spasms of fore limbs, T. 37.8°, R. 66. At 6.50 P. M., clonic jerking spasms involving both fore and hind limbs, head and neck — twenty jerks counted in half a minute. At 6.55 P. M., convulsive movements of hind limbs and gasping respiratory movements of lower jaw and neck. At 7 P. M., respiration very irregular and shallow, with occasional deeper breathing; T. 37.8°, R. 99. At 7.10 P. M., convulsive spasms of head and neck connected with inspiratory phase, but not occurring with every inspiration; feet and limbs extended; pressure on abdomen produces no movement; the lumbar and abdominal muscles constantly undergo rapid clonic contractions. At 7.15 P. M., respiration is very irregular with long sighing inspirations at intervals; no corneal reflex; pupils moderately dilated; no movement of the limbs in response to pricking or pinching. At 7.22 P. M.,

the respirations have almost ceased except for spasmodic inspiratory efforts; clonic spasm in hind limbs; heart beating strongly. At 7.24 P. M., respirations have ceased, the heart beat for a period of one minute and a half longer; temperature at death, 37.8° C.

Experiments on Goats.

These experiments were performed upon two full-grown goats about twelve months old from which the mammary glands had been removed some months previously in connection with other work, and upon two kids born from these in the laboratory. It was found that complete removal of the suprarenals in the goat is not necessarily fatal, and that where fatal the period of survival after removal of the second gland is longer than in the cat. Further, the clonic spasms which were so conspicuous a symptom in cats dying from suprarenal removal were entirely absent in the goats, which succumbed to the second removal. But in the goat, as in the cat, extreme muscular weakness and very rapid and shallow respiration were prominent symptoms.

These statements are supported by the following extracts from the protocols of the four experiments: —

Experiment I. — Brown kid; left suprarenal removed April 26, 1900, three hours after birth. Weight of gland, 0.137 gm.; weight of animal, 1.8 kg. The animal recovered rapidly from the operation, was afterwards fed regularly on milk from the bottle, and had begun to live partially on hay at time of second operation.

The right suprarenal was removed June 19, at 12.45 P. M. (interval between operations thirty-four days); weight of gland, 0.272 gm.; weight of animal, 4.5. kg. The animal rapidly recovered from anæsthetic and operative shock, and walked about the room a few hours after the operation. It was fed with milk the next morning (June 20) at 7.15 A. M., and drank about 2 ozs. It also ate a considerable amount of hay. It was fed again at 11.30 A. M., drinking 3½ ozs. of milk, and continued to eat hay. Again at 5.45 P. M., it drank 3½ ozs. of milk.

The temperature was taken as follows, after the operation: June 19, 10.45 P. M., 40.1° C.; June 20, 2.30 A. M., 40.2° C.; 5.10 A. M., 39.9° C.; 8.15 A. M., 40° C.; 10.10 A. M., 40° C.; 11.50 A. M., 40° C.; June 21, 12.10 A. M., 40° C.; 8.30 A. M., 39.5° C.; 1.50 P. M., 39.5° C.

After 9 A. M., June 21, the animal began to show marked muscular asthenia, and would not move unless forced to do so. The respiration became very rapid, and the heart slow and irregular. Gradually the animal became more

apathetic, and could scarcely be induced to rise. At 3.45 P. M., the respiration was 48 per minute and very shallow. At 5 P. M., the animal could not be induced to stand on its feet; respiration very shallow and at the rate of 60 per minute; heart irregular and slow. An injection of 5 c.c. of strong extract of ox-suprarenal into the peritoneal cavity seemed to cause a temporary recovery. The respiration slowed, the heart-beat became more rapid and regular, and the animal walked about the room. A quarter of an hour later the animal lay down again, but could get up and walk when disturbed. The respiration fell after the injection to 47 per minute, but at 6.45 P. M. it had again increased to 60 per minute. From this time onward the muscular weakness gradually increased; at 11.20 P. M., 1.5 C.C. of suprarenal extract was given, but this dose did not cause any material change in the animal's condition. The animal was left for the night at 11.30 P. M., and was found dead at 6 A. M. the next morning.

A post-mortem examination showed both wounds quite clean; no peritonitis; slight adhesions around wounds and between kidney and liver on one side, and kidney, spleen, and liver on the other. Abdominal organs all apparently normal. Stomach contained some coagulated milk and partially digested hay. In the thorax the lungs were almost normal, showing but a slight amount of congestion; the heart appeared somewhat enlarged for the size of the animal, but contained no ante-mortem clot.

The urine and extracts of various tissues of the animal (muscle, lymphatics, spleen, kidney, liver and blood) were examined chemically for suprarenal chromogen with negative results. Upon being injected into a cat they gave normal results of such tissues on blood pressure, showing the absence of any active suprarenal material in these tissues.

Duration of life in complete absence of suprarenals sixty to sixty-six hours.

Experiment II. — Black and white kid; right suprarenal removed immediately after birth, May 15, 1900. Rapid recovery; fed on milk and afterwards on hay. Left gland removed June 19 (interval thirty-five days) at 2.30 P.M. The recovery was normal, the animal walking about a few hours after the operation. It drank 2 ozs. of milk next morning at 7.15 A.M.; 3½ ozs. at 11.30 A.M.; and 7 ozs., that is a full meal, at 5.45 P.M. Throughout the day it also ate a considerable amount of hay.

The temperature was taken at short intervals throughout the night and following day and fell from 40.8° C., on recovery from anæsthesia, to a minimum of 39.7° C.; from this it rose again to 40.1° C., and maintained that temperature until near death on the afternoon of June 21.

At 11 A.M. on the 21st, it was first noticed that the animal had muscular weakness, could not stand up, and had a rapid and shallow respiration. The temperature was taken from this time onward at short intervals with the following results: — 11.10 A.M., 39° C.; 12.10 P.M., 39° C.; 1 P.M., 38.7° C.; 1.50 P.M., 38° C.; 3 P.M., 38.2° C. The respiration at 3 P.M. was 64 per

minute, *i.e.*, about double the normal rate; at 3.20 P.M. it was 72 per minute, and at 3.30 P.M. the animal was observed to be dead. Death occurred very quietly; although we were sitting beside the animal and had counted the respirations a few minutes before, we did not observe that it was dying. This shows that there could have been no muscular struggle.

The chief symptoms observed were profound muscular asthenia and exceedingly rapid and shallow respiration. Length of life after removal of second gland forty-nine hours.

The post-mortem showed both wounds clean; no peritonitis, no traces of suprarenals or accessories, no chromogen in urine or tissues; heart apparently enlarged and full of blood on both sides; no ante-mortem clotting; lungs normal; only assignable cause of death, suprarenal removal.

Experiment III. — Brown goat; right suprarenal removed May 23, 1900; rapid recovery, ate hay next morning and was sent out to feed until second operation.

Left suprarenal removed June 19 (interval twenty-seven days), at 3 45 P.M. Animal recovered rapidly from the anæsthetic and walked about the room. It drank water at 5.18 A.M. next morning and ate hay. Temperature on recovery from anæsthetic 40.4° C.; 5.10 A.M., June 20, 40° C. and remained stationary at this temperature during next day and night. The animal had a normal appetite on the three or four days following the operation but was somewhat apathetic. June 26 (seven days after the operation) it practically stopped eating and evidently was weak especially in the hind limbs and could rise only with an effort. The respiration was more rapid and shallow, and this condition became exaggerated after any muscular effort; thus, making the animal take a few steps increased the respiration to over 60 per minute. This condition became more marked, and at 11 P.M. on June 27, when the animal was unable to stand it was given a subcutaneous injection of 10 c.c. of a strong extract of ox-suprarenal, which, however, produced no noticeable effect. It was next examined at 2 A.M. on June 28, and was then found dead, with the head and neck in the position of opisthotonus and rigor mortis strongly developed.

A post-mortem examination made at 9 A. M., June 28, showed that rigor had already completely disappeared. Both wounds on the peritoneal surface were clean and the membrane had a normal glistening appearance. Behind the peritoneum, on the side first operated upon, there was a small caseous mass about two centimetres in diameter, and enclosed completely in a fibrous capsule. This must have formed about five weeks previously, after the first operation, and as it was completely shut off by its capsule of fibrous tissue and adhesions from the peritoneal membrane, we do not believe it can be responsible for the death of the animal; from the temperature before death, there did not appear to be any general septicæmia, and we are inclined to think that death must have been due to absence of the suprarenals. No traces of suprarenals or accessories were found. The tissues and urine contained no

chromogen. The abdominal organs all appeared to be normal. The lungs were normal. The heart was distended with blood on both sides, but contained no ante-mortem clot.

Chief symptoms, muscular weakness, apathy and rapid respiration. Period of survival without suprarenals, eight days and seven hours.

Experiment IV. — Black and white goat; left suprarenal removed May 23, 1900. The animal rapidly recovered and fed on hay next day. It was sent out to grass until second operation.

Right suprarenal removed June 19 (twenty-seven days' interval) at 5.15 P. M. The animal had the usual rapid recovery and ate hay and drank water next morning at 5.15 A. M.; from then onward appetite was normal. Temperature after recovery from anæsthetic, 40° C.; it afterwards fell to 39.5° C. and then slightly rose to 39.7° C. The animal was fed on hay in the laboratory until June 27 when it was taken to grass. From this date until July 11 the animal remained as far as could be observed in perfect health and ate grass and twigs greedily. It improved in condition and no trace of muscular weakness or other symptom developed. Circumstances prevented the experiment from being continued after July 11 and the animal was accordingly killed by chloroform and the body examined forthwith.

No trace of suprarenals could be recognized on either side, although a thorough examination was made. Certain small reddish glands looking like hæmal lymph glands together with the tissue, in the suprarenal region were removed, extracted chemically and tested for suprarenal chromogen with negative results; nor was there any suprarenal chromogen in the urine or other tissues. No accessory suprarenals were detected in connection with the sympathetic chain or the reproductive system and none were found elsewhere. The wounds were clean and were completely healed. They were shut off by adhesions from the peritoneum. There was also the usual amount of peritoneal adhesion near the wounds. All the other organs both in thorax and abdomen appeared to be quite normal.

This animal with both suprarenals completely removed survived therefore twenty-two days, and survival would apparently have been indefinite, for the animal, so far as could be observed, was unaffected by the loss of the glands. It is, of course, impossible to conclude, notwithstanding the most careful post-mortem examination, that the animal did not possess *somewhere* accessory suprarenals, which vicariously took on the functions of the removed glands. Still we are inclined to regard such a solution of the survival as improbable; certainly there were no glands visible to us at all resembling suprarenals in either thorax or abdomen, and nothing which gave a positive answer to the chemical test for the suprarenal chromogen. Any possible accessory glands must, therefore, have been either microscopic in size or situated outside the regions where their existence was to be expected.

Conclusions.

1. Complete suprarenal removal in the cat has in all our experiments been followed by a fatal result, the longest period of survival being just under five days.

2. In three out of seven experiments, extensive ante-mortem clotting in the right heart, superior vena cava, or pulmonary artery has been found, and in these cases the duration of life has been shorter than in those in which no such clots were found.

3. The chief symptoms observed in the cat after complete suprarenal removal were muscular weakness followed by extremely rapid, shallow, and irregular respiration, accompanied by rapid clonic muscular contractions affecting the whole system of skeletal muscles.

4. Death took place from respiratory failure in those cases in which it was observed, the heart continuing to beat for some time after respiration had ceased.

5. Removal of both suprarenals in the goat caused death in only three out of four cases.

6. The average period of survival in the three goats which succumbed was much longer than that in the cats.

7. No clonic contractions were observed in the goat at any period after suprarenal removal, the chief symptoms in this animal, in the three fatal cases, being extreme muscular weakness and rapid, shallow respiration.

A NOTE ON THE EXCRETION OF KYNURENIC ACID.

By WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

IN their paper on the excretion of kynurenic acid, Mendel and Jackson showed that substance to be a direct product of proteid catabolism. They found, further, that excretion of kynurenic acid accompanied accelerated proteid decomposition, whether this condition was brought about by fasting, or the ingestion of proteid food in quantities largely in excess of the needs of the body, or through the action of drugs. These observers also noted that, in conditions of ordinary nitrogenous equilibrium, the kynurenic acid in the urine was greatly diminished or might be entirely absent.[1]

The author, in repeating recently some of Mendel and Jackson's experiments, determined the excretion of kynurenic acid (1) during periods of nitrogenous equilibrium; (2) when proteid catabolism was stimulated, by chemical dosage as well as by excessive ingestion of proteid substance; and (3) when proteid catabolism was diminished by the lack of food. The animal, a healthy mongrel bitch, weighing 15 kilos, was confined in a cage suitable for metabolism work and given daily, at 9 A. M and 6 P. M., in two equal portions, a diet of 250 gms. of hashed meat,[2] 50 gms. of cracker meal, 40 gms. of lard and 700 c.c. of water, containing a total of 9.854 gms. of nitrogen.

The experiment lasted twenty-four days and was divided into three periods. Throughout the first period, of seven days, normal conditions prevailed and the dog was in almost perfect nitrogenous equilibrium. During the second period, ten days, the animal was given

[1] MENDEL and JACKSON: This journal, 1898, ii, p. 190. See also, MENDEL and SCHNEIDER: Proceedings of the American Physiological Society. This journal, 1901, v, p. ix.

[2] The hashed meat was prepared in bulk, freed from surplus moisture and kept in bottles, in a cold storage room, the frozen condition maintaining constancy of composition.

several large doses of tellurous oxide, a substance which not only causes slight stimulation of proteid catabolism, but likewise induces vomiting and loss of appetite.[1] In the third period, of seven days, normal conditions were present once more and equilibrium was restored.

On the morning of the second day of the dosage period, when the greatest amount of tellurous oxide was administered (0.5 gm. with the morning meal), all of the food given with it was vomited immediately. The second half of the daily portion of food was vomited in the evening also, so that no food was retained that day.[2] On the following day twice the usual amount of food was given. All of it was eaten and retained. For the remainder of the dosage period no gastric disturbances were induced and nitrogenous equilibrium was restored.

The experimental data[3] in this connection are given herewith in the table on the opposite page.

Nitrogen was determined by the Kjeldahl process; uric acid with Ludwig's,[4] kynurenic acid with Capaldi's,[5] methods. Uric acid was determined in combined urines, which were preserved with powdered thymol; the figures in the tables were recorded on the last days of each separate combination. The nitrogen of the daily food was 9.854 gms. The "total nitrogen balance" includes the nitrogen of the fæces and hair. The nitrogen of the vomit of the ninth day (10.335 gms.) was subtracted from the ingested nitrogen of the period in striking the balance. The total nitrogen in the fæces of the three periods was 2.374, 5.154 and 3.291 gms., respectively; in the cast off hair it was 1.054, 1.232 and 1.184 gm. The amount of tellurous oxide given on the first day of the dosage period was 0.5 gm., on the second, 0.75 gm.; on each of the third and fourth, 0.25 gm.; during the remainder of the period, 0.1 gm. per day. Indican, determined by the Jaffe-Stokvis test,[6] was present in the urine of each period.

[1] Mead and Gies: This journal, 1901, p. 147.

[2] The quantity of nitrogen in the vomit slightly exceeded that of the daily food, showing that none of the latter had been retained. The excess of nitrogen in the vomit came from gastric mucus.

[3] These results were presented informally at the last annual meeting of the American Physiological Society.

[4] Neubauer und Vogel: Analyse des Harns, zehnte Auflage, 1898, p. 820.

[5] Capaldi: Zeitschrift für physiologische Chemie, xxiii, p. 92.

[6] Neubauer und Vogel: *Ibid.*, p. 166.

1. Fore Period.				2. Dosage Period.					3. After Period.			
Day. No.	Nitrogen. Grams.	Uric acid. Gm.	Kynurenic acid.	Day. No.	Nitrogen Grams.	Uric acid. Gm.	Kynurenic acid. Gm.	Remarks.	Day. No.	Nitrogen. Grams.	Uric acid. Gm.	Kynurenic acid.
1	10.421		trace	8	8.982		none	Usual quantity food eaten	18	9.002		none
2	10.013		trace	9	3.654		0.046	All food vomited	19	9.434		none
				10	13.117		0.180	Double quantity food eaten				
3	9.403		none	11	10.031	0.284	0.131	Usual quantity food eaten	20	8.831		none
4	8.921	0.242	none	12	12.831		0.085	Usual quantity food eaten	21	9.324		none
				13	9.982		trace	Usual quantity food eaten				
5	9.410		none	14	9.674		none	Usual quantity food eaten	22	9.238		none
6	8.960		none	15	8.361		none	Usual quantity food eaten	23	8.597		none
				16	8.904		none	Usual quantity food eaten				
7	8.231	0.199	none	17	9.206	0.328	none	Usual quantity food eaten	24	8.768	0.468	none
Totals	65.359	0.441	trace	Totals	94.752	0.612	0.442		Totals	63.194	0.468	none
Daily averages	9.337	0.063		Daily averages	9.475	0.061		...	Daily averages	9.028	0.067	
Total nitrogen balance, + 0.191				Total nitrogen balance, — 3.079.					Total nitrogen balance, + 1.309			

The results of this experiment agree entirely with those obtained by Mendel and Jackson. It will be seen from the table that, excepting traces at the very beginning of the experiment when the dog was about to enter into equilibrium, kynurenic acid was eliminated only during the second period and then only on the days when the physiological balance was upset by the circumstances attending tellurium dosage. When the animal drew upon its own store of proteid, as it certainly did on the day of vomiting, kynurenic acid in small quantity was excreted for the first time. On the following day, when fed more than enough to satisfy its immediate needs, kynurenic acid was again eliminated. On the two succeeding days excretion of kynurenic acid continued; but it failed to appear when equilibrium was restored.

That the dog was in almost perfect nitrogenous balance during the second half of the dosage period (five days), when, with the exception of the trace on the thirteenth day, no kynurenic acid was eliminated, is evident from the following summary:

Nitrogen excreted:		
Urine	46.127	
Fæces [1]	2.577	49.320
Hair [1]	0.616	
Nitrogen ingested		49.270
Nitrogen balance		— 0.050

From these figures it is also clear that the increased nitrogenous catabolism, represented by 3.079 gms. of nitrogen (the "total nitrogen balance"), occurred in the first half of the period, during four days of which kynurenic acid was eliminated in appreciable quantity. These results indicate, further, that when nitrogenous equilibrium is completely upset by vomiting, it may sometimes be quickly restored by proper quantitative feeding.

It seems worthy of note, in this connection, that intestinal putrefaction, as indicated by the constant presence of indoxyl in the urine, was normal throughout the experiment. This, since kynurenic acid was excreted only when metabolism was disturbed, suggests, of course, that formation of this substance may occur independently of putrefactive changes in the intestine.[2] It certainly may be entirely absent when putrefaction is quite marked.

[1] The figures for nitrogen of fæces and hair represent one-half of the totals for the period. The quantitative elimination of each was constant daily, so that the above amounts are almost exact values.

[2] See MENDEL and SCHNEIDER: *Loc. cit.*

In conclusion, attention may be drawn to the fact that uric acid was eliminated in constant quantity throughout the experiment and that, therefore, kynurenic acid did not replace it. Excretion of the latter occurred independently of elimination of the former. The results recorded here confirm the observations of Solomin,[1] and also those of subsequent workers in this connection.

[1] Solomin: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 497.

ARE THE CONTRACTIONS OF THE LYMPH HEARTS OF THE FROG DEPENDENT UPON CENTRES SITUATED IN THE SPINAL CORD?

By ANNE MOORE.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

IN a recent article[1] I have shown that the lymph hearts of the frog will beat normally for many hours if excised and placed in certain salt solutions. The most efficacious solutions were found to be

$\frac{1}{8}n$ NaCl.
100 c.c. $\frac{1}{8}n$ NaCl + 4 c.c. $\frac{2}{8}n$ $CaCl_2$.
100 c.c. $\frac{1}{8}n$ NaCl + 4 c.c. $\frac{2}{8}n$ Na_2SO_4.

If kept at a favorable temperature (10–15° C.) hearts placed in these solutions continue to contract from twenty-four to sixty-seven hours.

Aside from its relations to the effects of ions upon rhythmical contractions, the fact is of interest from another point of view. For many years the contractions of the lymph hearts of the frog have been supposed to depend entirely upon the action of automatic nerve centres situated in the spinal cord. This idea arose from the observation made by Volkmann,[2] that if the spinal cord is destroyed or if two spots in the cord in the region of the sixth and eighth vertebræ are injured, the hearts stop beating. On the basis of this observation was assumed the existence of centres located in the spinal cord. Volkmann found, however, that after a time the lymph hearts of a frog whose spinal cord had been destroyed might begin to beat again. This was out of harmony with the idea that controlling automatic centres are present in the cord, and was difficult to explain. According to Volkmann, there are three suppositions which may account for the normal contractions of the lymph hearts: (1 their contraction may be entirely independent of nerves; (2) automatic centres may exist in the hearts themselves; (3) automatic centres may exist in the spinal cord. The first of these suppositions was not seriously considered either by Volkmann or by other

[1] MOORE, ANNE: This journal, 1901, v. p. 87.
[2] VOLKMANN: Müller's Archiv, 1844, p. 419.

investigators of his time. Volkmann, followed by Eckhard,[1] Schiff,[2] and Priestley,[3] believed that the temporary cessation of beats after the destruction of the cord effectively disproved the presence of centres in the hearts themselves. These observers, therefore, held to the idea that automatic centres exist in the cord, in the region of the third and sixth[4] vertebræ. They held that the contractions observed after the separation of the lymph hearts from the centres were different in character from the original contractions, and Volkmann explained their occurrence by attributing to nerves a strength-storing power.

Goltz[5] and Waldeyer[6] disagreed with this opinion, locating the centres in or near the hearts themselves. In an experiment performed by Goltz, the tenth spinal nerve of a frog was severed on one side. The posterior lymph heart on this side stopped for a time and later resumed beating. The frog was kept alive and a week afterward the spinal cord was destroyed. The other three hearts stopped beating; but the first continued to beat even when removed from the body. This he considered a direct proof that the motion of the hearts is not dependent upon the spinal cord. As is the case with the thoracic heart, Goltz found that tapping the intestine caused the lymph hearts to stop and then begin again more quickly (Klopfversuch). If the cord is destroyed, this effect is not produced. From this Goltz concluded: (1) that a nervous motor organ is present in the hearts; (2) that a constantly active inhibitory centre is present in the optic lobe; (3) that reflex inhibition may be caused by impulses passing from the abdominal viscera through the sympathetic to the medulla and thence through the fibres in the spinal cord to the lymph hearts. Suslowa[7] and others confirmed these last ideas. Waldeyer accepts the first conclusion and locates a motor organ in

[1] Eckhard: Experimentelle Physiologie des Nervensystems, 1866, p. 259; Zeitschrift für rationelle Medicin, 1849, viii, p. 211; Hermann's Handbuch der Physiologie, II, pp. 55, 73.

[2] Schiff: Zeitschrift für rationelle Medicin, 1850, ix, p. 259.

[3] Priestley: The journal of physiology, 1878, i, p. 1.

[4] This location is given by Priestley, who dissents from those who place the centre in the region of the eighth vertebra.

[5] Goltz: Centralblatt für die medicinischen Wissenschaften, 1863, pp. 17, 497; 1864, p. 691.

[6] Waldeyer: Zeitschrift für rationelle Medicin, 1864, 3d series, xxi, p. 103.

[7] Suslowa: Zeitschrift für rationelle Medicin, 1868, 3d series, xxxi, p. 224; Centralblatt für die medicinischen Wissenschaften, 1867, p. 832.

ganglion cells which he succeeded in finding in the pigment speck near the heart. He asserts that if this speck is removed or the ganglia destroyed, normal contractions cease and do not return.

Recently C. W. Greene[1] has expressed the opinion that the pulsations of the caudal heart discovered by him in the hag fish are dependent upon an automatic centre in the spinal cord. He bases his belief upon the fact that destruction of the cord causes a cessation of beats which he states are not resumed.

My own observations show that:

(1) In accordance with the statements of other observers, the lymph hearts stop beating for a time after destruction of the spinal cord. This is not surprising, but the fact does not prove that the hearts are dependent upon centres in the spinal cord for their power to beat rhythmically. It is unfortunate that the word centre should be bound up with the idea of a causal relation. Loeb[2] points out that a spinal cord centre is merely the anatomical location of the origin of fibres supplying the organ in question. Stimulating or destroying such centres naturally modifies the activity of the organ.

(2) Excised lymph hearts possess the power of beating automatically. That some of the authors mentioned were not able to convince themselves of the fact may be attributed to their failure to surround the heart by the right solution. As the lymph heart is as capable of automatic activity as the thoracic heart, there is no more reason for assuming that the activity of the lymph heart depends upon centres situated in the spinal cord than there is for assuming the dependence of the blood heart upon such a centre.

[1] Greene: This journal, 1900, iii, p. 366.

[2] Loeb: Comparative physiology of the brain and comparative psychology, 1900, pp. 134–149.

OBSERVATIONS ON THE CHANGES IN BLOOD-PRESSURE DURING NORMAL SLEEP.

By C. E. BRUSH, Jr., and R. FAYERWEATHER.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

WITH the view of ascertaining the changes which occur in the blood-pressure of man during the period of normal sleep, a series of observations was made, the results of which are herein described. The results obtained give curves which agree so closely in their main points that we feel justified in presenting them as representing the general character of the course of the arterial pressure in the normal sleep of a healthy individual, the pressure being measured in the arteries of the right hand.

Many observations on blood-pressure have been made by different investigators with various forms of apparatus and under different conditions of the body. Some of these observations were made during sleep, but no reference has been found in literature to any series of records that were taken during the whole period of normal sleep.

Kiesow[1] gives one reference to an experiment made by him, in which a record was taken on the subject before going to sleep and another while sleeping. He gives the pressure as 80 mm. Hg while the subject was awake and states that the record made during sleep showed a small lowering of pressure, but he gives no figures. The apparatus used by him was Mosso's sphygmomanometer.

Hill,[2] working with the Hill-Barnard sphygmometer, has studied the effect of sleep upon the blood-pressure, but he speaks of making only one observation during each period of sleep. His results, which are the averages of a large number of observations, show a fall of 2–5 mm. Hg within an hour and a half after going to sleep and then a further fall of 3–5 mm. in the record taken immediately after awaking, the subject being engaged in mental work. No curve is given. His results contradict those obtained in the present experiments, which in every case show that the pressure after awaking is as much as 10–24 mm. Hg higher than before going to sleep.

[1] Kiesow: Archives italiennes de biologie, 1894, xxiii, p. 198.

[2] Hill: Journal of physiology, 1898, xxii, p. xxvi.

Colombo,[1] also working with Mosso's instrument, has determined the diurnal curve of pressure under two different conditions, — one, in which the hours of his meals were so arranged that all vaso-motor changes due to digestion had passed off before the records were taken; and a second, in which the meals were taken at the regular hours. In each case the observations were made at intervals of half an hour. He makes no reference, however, to the time that elapsed between his sleep and the time of taking the records.

Von Wagner[2] has taken records with Gärtner's[3] tonometer on sleeping patients, chiefly paralytics and epileptics. He states that the record after awaking showed an increased pressure of 20–30 mm. Hg and in one case an increase of 40 mm. In the latter case paraldehyde had been administered to the patient before going to sleep. None of his records was made on healthy subjects.

Walden,[4] using his modification of Mosso's apparatus, made a series of sphygmomanometric records on a subject during hypnotic sleep, the period of sleep lasting from three to five hours. The observations were made at intervals of fifteen minutes. The resulting curves were combined into one average curve, the general course of which was horizontal up to the time of awaking, when a marked increase in pressure took place.

Apparatus and Methods.

In the present experiments some difficulty was at first experienced in getting the subject to go to sleep. Toward the end, however, as the persons experimented upon became accustomed to the new conditions there was little, if any delay. In the first experiments Mosso's sphygmomanometer, as designed for use with the fingers, was employed. This instrument is constructed on the principle that the pulsations of the arteries exhibit their greatest amplitude when the mean pressure in the arteries is counterbalanced by an equal pressure on the outside. Our apparatus was constructed after the model described by Mosso,[5] the only difference being in certain minor points, especially the placing of the fingers in the metal tubes. For this latter purpose thin rubber finger cots were used. These were

[1] Colombo: Archives italiennes de biologie, 1899, xxxi, p. 345.
[2] v. Wagner: Wiener klinische Wochenschrift, 1899, xii, p. 717.
[3] Gärtner: *Ibid.*, p. 696.
[4] Walden: This journal, 1900, iv, p. 124.
[5] Mosso: Archives italiennes de biologie, 1895, xxiii, p. 177.

inserted into the metal tubes and their ends were reflected over the outside of the tubes, the latter being protected by heavy rubber bands, to prevent the metal edges from tearing the cots. Rubber stoppers were then cut out to fit the fingers and inserted into the finger cots as they lay in the metal tubes. In addition, metal guards were screwed down against the edges of the rubber stoppers to prevent them from being forced outward when the pressure inside the tubes was raised. The fingers were inserted through openings in the metal guards and the rubber stoppers as far as the second phalangeal joint. The rubber stopper rings fitted snugly to the fingers and the hand was kept in position by supports, similar to those described by Mosso, which were pressed against the back of the hands. The subject slept on his back with the sphygmomanometer resting on his abdomen. Owing to the discomfort caused by having both hands rigidly fixed, he was very restless and consequently easily awakened by any slight noise. The apparatus also offered a large source of error, inasmuch as the sleeper could change the level of zero pressure by drawing the apparatus upward on his chest or by turning on his side. But the chief difficulty encountered was a steady decrease in the amplitude of the pulse wave from the time of going to sleep until 3 or 4 A. M., when the amplitude was so small that it was impossible to determine the level at which the maximum excursion of the needle was obtained.

In order that the sleeper might rest more quietly, Walden's[1] modification of Mosso's apparatus was tried. In this instrument only one hand is necessary. It consists of two glass tubes, about 3 cm. in diameter and 15 cm. long, drawn out at one end to a diameter of 1.5 cm. These are mounted on a wooden base. Finger cots are placed at the large ends and the other ends are connected by means of a Y tube with the cross-piece of a T tube. The shaft of the T tube is connected with the pressure reservoir, the other end of the cross-piece being attached to an inelastic rubber tube leading to the mercury manometer. The apparatus was swung by a chain from the ceiling. It possessed an advantage over the other instrument in that the sleeper rested more quietly and his movements did not affect the level of zero pressure. But it also proved unsatisfactory because toward morning the amplitude of the pulse wave became so small that a determination of arterial pressure was not possible. To prove that the decrease was not due to mechanical

[1] WALDEN: This journal, 1900, iv, p. 124.

occlusion of the arteries from having the hand in the apparatus for so long a time, the subject on awaking put his other hand in the apparatus. This failed to give any greater amplitude, showing that the decrease was referable to some change in the peripheral blood-vessels themselves. The difficulty seems to lie in the fact that in these forms of apparatus in which the pulse is measured in the ends of the fingers we are dealing with small arteries which probably participate directly in the vaso-motor changes. If these arteries begin to constrict toward morning, as seems probable from these and other experiments, the result would be a diminished amplitude of pulsation for two reasons; first, because the increased tone may give a less extensible wall, and second, because the pulse would be measured more on the capillary side of the peripheral resistance, and would therefore be actually diminished in force. It would seem, in fact, that the finger sphygmomanometer cannot be used to determine variations in arterial pressure in which the finger arteries themselves participate, owing to the fact that during vaso-constriction of these vessels the pressure measured would not be a true index of the general arterial pressure. While the latter was rising the finger manometer might indicate a fall, owing to the fact that the pressure was being measured on the capillary side of the resistance.

After a sufficient number of trials on two subjects to prove the inefficiency of these two forms of sphygmomanometer for the work in question, it was decided to use some modification by which a record could be obtained from the larger arteries of the hand and wrist. For this purpose we made use of a plethysmograph capable of holding the hand and lower part of the forearm, arranging it however to act as a sphygmomanometer in which the pressure could be raised or lowered at will and the pulsations and pressure could be recorded by a mercury manometer. In this form it is simply a modification of Mosso's instrument. The essential part of the apparatus is illustrated in Fig. 1, and may be described as follows. A thick-walled glass cylinder about 30 cm. long, with an internal diameter of about 9 cm., is open at one end to admit the hand and wrist which are secured there in a manner soon to be described. The other end is drawn out into a short neck in which is inserted a rubber stopper. Through this stopper a brass tube (A) passes into the interior of the cylinder. A flange prevents its entering beyond a certain point. The inner end of the tube is threaded, and on this is screwed a brass nut. To the inner surface of the nut is riveted a brass

wire bent into the shape of a heart. Consequently, when the nut is screwed tightly on the tube, the two flaring sides of the brass wire press against the neck of the cylinder, into which they are too large to pass, and so they hold the rubber stopper firmly in place, while the flange beyond the stopper prevents the tube from being drawn in. Fastened to this piece of brass wire are two rubber rings large enough to admit a man's finger. When the subject is put into the apparatus he passes a finger through each of these rings and then, flexing his finger, pushes in his arm until his closed fist rests against the end of the cylinder. In this position he cannot release his grip on the

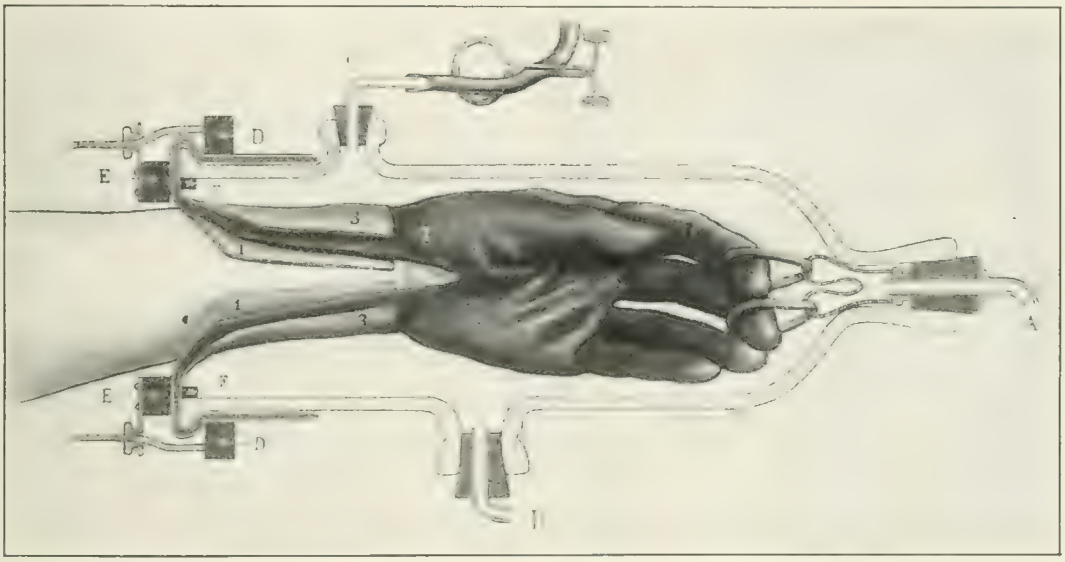


FIGURE 1. — Hand-plethysmograph adjusted for use as a sphygmomanometer.

A, tube to manometer. B, inlet from pressure flask. C, waste outlet. D, large hard-rubber collar, seen in cross-section. E, small hard-rubber collar, seen in cross-section. F, hard-rubber ring around wrist, seen in cross-section. 1, inner sleeve of rubber dam. 2, rubber glove. 3, outer sleeve of rubber dam.

rings during sleep nor can his arm be forced out of the apparatus when the external pressure on the hand is raised. This method of retaining the arm within the glass cylinder is simple and more comfortable to the subject than the devices previously described by Shields[1] and by Howell[2] in their plethysmographic experiments. The outer end of the brass tube is connected with a long piece of lead or inelastic rubber tubing leading to a mercury manometer.

In the lower surface of the cylinder, at about the middle, is a neck into which a rubber stopper is wired. Through the stopper passes a

[1] SHIELDS: Journal of experimental medicine, 1896, i, p. 74.

[2] HOWELL: Journal of experimental medicine, 1897, ii, p. 313.

short elbow of brass tubing (B) which communicates with a pressure flask swung from a pulley in the ceiling. The bottle, filled with water, constitutes the head of pressure, which is varied by adjusting the bottle at different heights. During an actual observation of the pulse the tube from this bottle is shut off by a pinchcock to prevent a loss of pulse from the expansion of the rubber tubing.

About 7 cm. from the large end of the cylinder, in the upper surface, is still another neck (C) in which a rubber stopper and short glass tube are similarly fixed. From this a rubber tube passes into a jar for waste water. When not in use, this tube is shut off by a pinchcock at its point of union with the glass tube. By means of a glass stopcock in the tube connected with the manometer, the communication of the latter with the cylinder may be shut off, and water may be passed from the reservoir through the cylinder and out through the waste tube. This is done in filling the cylinder and also whenever it is necessary to raise the temperature in the cylinder by a fresh supply of warm water.

The cylinder is hung from the ceiling by a chain passing over a pulley and is adjusted at a height convenient for the sleeper. The hand and arm are introduced into this glass cylinder in such a way as to be held rigidly and without leakage when the pressure in the cylinder is raised by means of the pressure flask. To accomplish this a comfortably fitting sleeve of thick rubber dam (1) is drawn over the forearm from the wrist-joint upwards for about 10 cm. A thin rubber glove (2) with a long gauntlet is then fitted to the hand and the gauntlet is pulled up the arm so as to lie over the sleeve of rubber dam. Another sleeve of rubber dam (3), the same length as the first but of slightly larger diameter, is then drawn over the glove. This method of using a glove in plethysmographic work is the same as that described by Walden [1] in the paper referred to above. It has proved very convenient, and does away with one of the most serious difficulties in plethysmographic work. The hand is next passed through a hard-rubber ring (F) which completely fills the mouth of the cylinder and fits comfortably around the forearm. Two fingers are passed through the rubber rings at the opposite end of the plethysmograph, and the hard-rubber ring at the wrist is pressed firmly into place so that it is flush with the mouth of the cylinder. The two sleeves of rubber dam with the gauntlet between them are then reflected over the edges of the cylinder upon its outer surface (see

[1] Walden: This journal, 1900, iv, p. 124.

Fig. 1). They are held there partly by their own elastic tension; but two collars, such as were used in Shields's [1] experiments, are used to further secure them. The larger of these (D) is a hinged hard rubber ring, wound with muslin, fitting tightly over the reflected bands, and pressing them firmly against the raised glass rim at the mouth of the cylinder. The other collar (E), of like design, encases the forearm and presses the bands against the end of the cylinder. In order that this pressure may be effective, this collar is provided with two small brass rings, one at each side, through which pass two brass uprights projecting from the larger collar. These wires are threaded and nuts are screwed on them by means of which the second collar may be drawn firmly against the end of the cylinder. For sphygmomanometric work it was found necessary to pack the small space between this collar and the forearm with soft muslin to prevent a distention of the reflected bands when the pressure within was raised.

The hand being thus adjusted, the subject lies down on a cot beside which the cylinder is hung, and the latter is fixed at a comfortable level, the elbow being supported by a sling attached to the chain which holds the cylinder. The manometer, kymograph, night-lamp and other apparatus stand on a low table beside the cot. Connection with the manometer is shut off by the stopcock, and warm water is run through the cylinder, the air passing out through the waste tube. When all of the air is driven out, the waste tube is pinched off and water is run through the outlet leading to the manometer. It is essential for obtaining a good pulse record that no bubbles of air shall be left in this tube. If the head of pressure be sufficient, a pulse wave will now be shown by the manometer. Experience has shown that it is best to keep the temperature of the water in the cylinder at from 33°–38° C.

Great care was taken in these experiments to avoid disturbing the sleeper in any way. Woollen shoes were worn by the observer, a night-lamp with a minimum amount of illumination was used, a dark curtain was interposed between the cot and the table, and the pulse was recorded on a noiseless drum.

In making the later observations, from which in the main the data reported were obtained, the subject always refrained from eating or smoking for at least two hours before the experiment. The first series of readings was made with the subject sitting on the cot, the cylinder

[1] Shields: Journal of experimental medicine, 1896, i, p. 74.

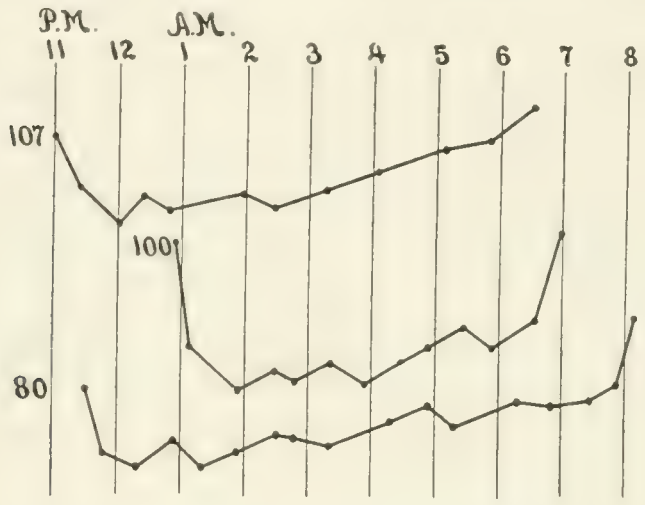
being at the level at which it was to be kept during the night. A second series was recorded about fifteen minutes after the subject lay down, while he was yet awake. During the night a series of readings was taken every half hour as a rule, and after each observation warm water was run through the cylinder. At each observation the pressure was gradually raised and successive readings of the pulse were taken at each change until a maximum amplitude of the pulse was obtained, and a further increase in pressure produced a decrease in the excursion of the recording needle. After each change of external pressure an interval of 10–15 seconds was permitted to elapse, allowing the pulse to accommodate itself to the new condition. In the later experiments readings were made only while the pressure rose, as it was found that prolonged pressure was liable to make the subject restless, or to awake him. Whenever the subject awakened, he made the fact known, and if the observer was in the room at the time the hour of awaking was noted. After the earliest experiments, however, it was comparatively seldom that the subject awoke during observations, while the ability to go to sleep quickly was so marked that difficulty was often found in keeping him awake for the first two records. On his awaking in the morning his pulse was recorded when he was in the reclining position, and again fifteen minutes after he sat up.

Results.

The general character of the results obtained is indicated by Fig. 2, which gives the plotted curves for the three best records obtained. The legend to this figure explains the curves with sufficient clearness, and attention may be directed at once to the points of general interest. It will be noted that in the beginning of each experiment the first observation was made with the subject in the sitting posture, and the second observation after he lay down but while still awake. This change from the sitting to the reclining position and the reverse change at the end of the experiment are always accompanied by a considerable change in arterial pressure as measured in the wrist arteries. This difference averaged about 23 mm. of mercury and it seems probable that it was due mainly to the hydrostatic influence of the column of blood in the pendent arm while in the sitting position. After lying down, a still further fall of pressure ensues upon going to sleep. The exact amount of this difference in pressure between the sleeping and waking condition

while in the recumbent position is difficult to state. According to our experiments it might vary between 4.4 and 13 mm. Hg, the average being 9.8 mm. The somewhat wide range of variation is to be accounted for, in part at least, by the fact that during the first observation made upon the subject lying down, preparatory to sleep, he was more wakeful in some experiments than in others, in some cases wide awake, in others drowsy and kept awake with difficulty.

Curves of blood-pressure during normal sleep.



Each millimetre on the ordinates = 2.9 mm. mercury pressure.

FIGURE 2. — Plotted curves of three sleep records.

The numbers at the beginning of each curve show mean arterial pressure in hand and wrist with the subject in the sitting position. The second point in each curve indicates the pressure with the subject reclining but still awake. The third point indicates the pressure at the taking of the first reading after the beginning of sleep.

Curve I. The next to the last point indicates the pressure at the last observation during sleep. The last point indicates the pressure after awaking, the subject being in a reclining position.

Curves II and III. In these curves the last point represents the pressure with the subject awake and sitting. The two preceding points correspond to the last two points of Curve I. All intermediate points represent pressures obtained from observations during sleep. The vertical lines represent intervals of one hour.

It is also uncertain whether the first observation made after the subject had gone to sleep invariably coincided with the point of lowest pressure. The general fact remains, however, that upon going to sleep the pressure falls to a certain minimum, which is reached some time within the first hour. After this the pressure begins to rise gradually, but with certain irregularities, until the awaking in the morning.

The plotted curves show a somewhat rhythmic rise and fall, but the periods of rising pressure are always the longer, so that in no case does the pressure fall as low as during the first hour, and the curve in general rises steadily. Toward morning the period of these alternations of pressure becomes larger and the variations smaller, resulting in a less broken rise in the curve. The first pressure recorded after awaking in the morning is higher in every case than that noted at any time during the night, and is invariably higher than the corresponding pressure recorded before sleep began, the subject being in the reclining position. As in the evening, the change from reclining to sitting posture has a marked effect upon the pressure. The fall of pressure in passing from the waking to the sleeping state has already been noted. In the morning, the change from sleep to wakefulness is always accompanied by a rise in pressure. In each series of records, this fall and rise are very nearly, if not quite, equal in value. Although no complete records throughout the night could be obtained from observations made with the first two forms of apparatus used, owing to the difficulties already described, the curves of the first part of these records closely agree with those under consideration.

The amplitude of the pulse wave shows a marked and invariable decrease throughout the night, but the curve is frequently interrupted by temporary changes in the opposite direction. These, however, are not of a striking degree. A considerable decrease in amplitude almost always accompanies the fall in pressure in the change from sitting to reclining at the beginning of the records. The greatest amplitude in almost all of the records appears in the first series of readings, the next greatest in the second series, that is, while the subject is still awake but reclining. In all other cases these variations show no constant relation to those in blood-pressure. In the morning the amplitude generally increases with the change from the reclining to the sitting posture.

On comparing these curves of pressure with the results obtained by Howell[1] for the changes in the volume of the arm it will be observed that in Howell's curves the point of greatest vaso-constriction was reached from one to one and a half hours after sleep began, and that in the present curves the point of lowest pressure is reached and passed within the first hour after the beginning of sleep. The

[1] Howell: Journal of experimental medicine, 1897, ii, p. 313.

periods of rise and fall in the pressure curves show some similarity to the rhythmic, secondary waves noted by Howell in his plethysmographic curves; but whether there is any other than an apparent relation between them we have not attempted to discover. The gradual rise of arterial pressure noted in our experiments agrees in a general way with the diminishing volume of the arm described by Howell, although the rise in our curves seems to begin more promptly than in his.

If the pressure curves recorded by us are compared with curves of intensity of sleep, they are found to agree most closely with those of Kohlschütter.[1] In general the similarity is this: the intensity of sleep is greatest and the arterial pressure lowest during the first half of the period of sleep. After this point the former decreases and the latter increases fairly regularly up to the moment of awaking. The intensity of sleep is greatest at the end of the first hour, at the time that the blood-pressure has reached its lowest point. In this respect the present curves do not agree with the curves of intensity of sleep obtained by Mönninghoff and Piesbergen.[2]

Summary.

Our conclusions and results may be briefly summarized as follows:

1. The blood-pressure as measured in the wrist arteries by means of the method of maximal amplitude of pulsations is lower in the late evening than in the early morning, when the external conditions (posture) are as nearly similar as possible.

2. The blood-pressure in the wrist arteries falls about 20 mm. of mercury when the subject passes from a sitting to a reclining position, the actual height of the hand remaining the same in both cases. This fall is mainly referable to the hydrostatic effect of the column of blood in the pendent arm.

3. During sleep the blood-pressure falls during the first few hours and then gradually rises up to the time of awaking. This rise is not regular, but is broken by long waves, although the general tendency of the pressure is toward a steady increase. On wakening in the morning the arterial pressure is greater than just before sleep, when the pressure is measured in the same (reclining) position.

4. With reference to the bearing of these observations upon theo-

[1] Kohlschütter: Zeitschrift für rationelle Medicin, 1863, xvii, p. 209; 1869, xxxiv, p. 42.

[2] Mönninghoff and Piesbergen: Zeitschrift für Biologie, 1883, xix, p. 114.

ries of sleep, it is important to emphasize the fact that the rise of pressure on awaking is not sudden. It is the culmination of an increase that begins to appear within an hour or two after sleep has begun. This fact would seem to indicate a progressive vaso-constriction occurring during most of the period of sleep, and to this extent it is in harmony with the so-called vaso-motor theories of sleep.

We desire to thank Dr. Howell and Dr. Dawson for frequent suggestions that have made our work easier and our results more accurate.

PHOTOTAXIS IN THE AMPHIPODA.

By SAMUEL J. HOLMES.

[*From the Zoölogical Laboratory of the University of Michigan.*]

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IN the present paper I have embodied the results of several experiments, performed chiefly upon Amphipods during the past summer at Wood's Hole, Mass. The experiments were undertaken primarily in order to throw light on two questions, the mechanism of the phototactic response, and the cause of reversal in the sense of phototaxis. A little light has, perhaps, been gained regarding the first question; but, while some of the conditions that bring about reversal of phototaxis have been ascertained, the facts discovered render the second problem somewhat more difficult than it seemed before.

Phototaxis in the Aquatic Gammaridea.

It seems to be a general rule that the aquatic Gammaridea are negatively phototactic. This was uniformly the case in the following species which comprise all the forms with which I have experimented: Corophium cylindricum (Say), Maera laevis (Smith), Unciola irrorara (Say), Hyale littoralis (Stimpson), Pontogeneia inermis (Kröyer), Gammarus mucronatus (Say), Gammarus locusta (L.), Melita nitida (Smith), Calliopius Rathkei (Zaddach), Ampelisca sp. (Smith), Autonoe sp. (Smith), Cerapus minax (Smith), Amphithöe

maculata (Stimpson), Amphithöe longimana (Smith), Amphithöe sp., Hyalella dentata (Smith), Microdeutopus grandimanus (Bruz.), an unidentified, probably new species of Jassa, and a new species, if not genus, of Calliopidae.

If specimens of Gammarus or Amphithöe be placed in a dish exposed to the light they immediately swim to the side furthest from the source of illumination. If the dish be turned they soon swim back to the other end. In lamplight they may be driven to any side of the dish by moving the lamp. If a single individual be watched it will be seen to struggle for a time, to move away from the light; it will then rest, for a longer or shorter period, only to resume its struggle later. In a dish containing numerous specimens of Gammarus locusta, which were taken from a fresh-water pond near Falmouth where they had become acclimated, it was noticed that individuals suddenly left the Ceratophyllum that was kept in the water and swam quickly to the negative end of the dish, where they darted actively about for a while in the endeavor to get away from the light; when an apparently chance movement brought them in contact with the Ceratophyllum again, they would remain. If there were no objects in the dish that appealed to the thigmotactic proclivities of the animals the majority were found at the negative end throughout the day; if there were such objects, however, the creatures were brought in contact with them in their random excursions, and here they remained until the phototactic impulse again drove them forth. In the dish of Gammarus locusta there was one group at the negative end and another in the Ceratophyllum, and individuals continually passed back and forth from one to the other.

Phototaxis in the Terrestrial Amphipods.

The behavior towards light of the terrestrial amphipods with which I have experimented stands in marked contrast to that of the aquatic species. The three species studied, Talorchestia longicornis, Orchestia agilis, and Orchestia palustris are all positive under ordinary conditions. An European species of Orchestia whose specific name was not mentioned was found by Delle Valle[1] to be strongly positive. It seems to be the rule that positive phototaxis prevails among the terrestrial amphipods, just as negative phototaxis prevails

[1] Della Valle: Fauna and Flora des Golfes von Neapel, xx Monographie, Gammarini, p. 122.

among the aquatic forms. The three species studied differ considerably in their reactions to light and will be separately described.

Talorchestia longicornis. — This species is very common near Wood's Hole, especially on sandy beaches. During the day it lies buried in the sand, generally a little above high tide mark, but never where the sand is very dry. It commonly inhabits places where the sand is dry on the surface, but moist (not wet) an inch or two below. Its places of concealment are indicated by holes in the sand. When dug out Talorchestias are usually sluggish and often curl up and remain motionless like many insects that exhibit the so-called death-feigning instinct. Sometimes they make a few jumps in the endeavor to escape, and then burrow in the sand. When placed in the sand they invariably burrow into it and remain quiet. At night they come out to feed, and may be found in large numbers actively running over seaweeds recently washed up by the waves.

The eyesight of Talorchestias is exceedingly good, as they can detect a person in the dark several yards away. The observer must crawl carefully up to these animals in order to study their habits. When alarmed they make for their burrows, which they can detect at a distance of several inches. Their alertness at night shows a marked contrast to their dazed and stupefied condition when dug out in the daytime. If a lantern be placed in their midst they are attracted to it in large numbers. When walking about over the seaweed individuals may be seen to stop as soon as they are affected by the light. They quickly turn and face the lantern, and then make straight towards it, either by walking or by a series of leaps. I have often made specimens brought into the laboratory at night follow a lamp carried about the room.

When specimens of Talorchestia are placed in a dish near a window they move quickly toward the light, many jumping vigorously against the side of the dish, others trying to crawl up the side. If the dish be turned about, they all scamper quickly toward the light again. The response is so marked that the creatures seem to be seized by some mad impulse. Their responsiveness continues in spite of long exposure, as they persist in making efforts to go towards the light during the whole day. For quickness, definiteness, and duration of response to light Talorchestia is probably unexcelled by any other known animal.

A very weak light is sufficient to elicit a marked positive response in this species. Experiments were tried in order to ascertain if strong

light would cause a change in the sense of phototaxis as it is known to do in many forms, but it was found that the stronger the light the more marked, if anything, was the positive response. When Talorchestias are exposed to direct sunlight, or to direct sunlight reinforced by light reflected into the dish by a mirror, they walk or hop to the positive end of the dish and struggle there until overcome by the heat of the sun. If a dish containing Talorchestias be left exposed very long to direct sunlight the specimens will be found dead in the end of the dish turned toward the sun.

Yet notwithstanding its marked or almost violent positive phototaxis, Talorchestia generally comes to rest in shady spots. It may seem at first somewhat singular that an animal should be positively phototactic and still evince a tendency to keep in the shade, but there is really no contradiction in this. The same combination of traits is, I believe, not uncommon among other animals. When put into an inclosure with objects casting a small amount of shade Talorchestias will be found to show a strong tendency to collect on the shaded instead of the lighter side of the objects. Often they come out and go toward the source of light; but if they again happen into a shaded retreat they are very likely to remain there. Talorchestias come to rest in shaded places apparently because there they are less stimulated. The effect of light is to force them into activity, and to compel them to go towards the source of illumination. Where there is a respite from this stimulus they remain relatively quiet. The animals do not go to the positive end of the dish in order to get from a darker into a lighter area; they will go to this end when it is made somewhat darker than the other by colored glass; Loeb has shown a similar reaction in several other animals that are positively phototactic. The behavior of Talorchestias naturally produces the impression that they prefer to rest quietly in the shade but are drawn out by the phototactic impulse apparently against their will and set going in the direction of the rays of light.

Orchestia agilis. — Orchestia agilis may be said to be normally positively phototactic, but under certain circumstances it may become strongly negative. It is not entirely a nocturnal animal in its habits, like Talorchestia. It keeps away from the light during the day under damp masses of seaweed on the beach, where it occurs in countless numbers. On turning over a mass of seaweed hundreds of these creatures may generally be seen hopping in a very lively manner in all directions. In my experiments on this species I have dealt with

large numbers — commonly several hundreds, — as it is very easy to obtain specimens in any desired quantity.

For a short time after the Orchestias are collected on the beach their behavior to light is far from uniform. If specimens are collected in an oblong glass dish and exposed at once to the sunlight a number will be found jumping towards the light, while most of the others will be jumping away from it. Comparatively few will be found between the two ends of the dish. The proportion of specimens at the two ends in lots collected at different times was found to be variable, the majority being sometimes positive and sometimes negative. In either case the specimens at the negative end gradually come over to the positive end, and, in the course of about half an hour, all the specimens become strongly positive and remain so. If specimens are collected in the morning they will remain positive all the rest of the day. The effect of strong light was tried, as in Talorchestia, and it was found that it had not the least tendency to make the specimens negative. Specimens in the direct sunlight remain positive as long as they retain life, going towards the light as long as they have strength enough to crawl. If specimens recently taken from under seaweed be exposed to direct sunlight those that were at first negative become positive very quickly. If other specimens taken at the same time and place be exposed to less intense light from a north window the transformation will be much slower. In either case, if the intensity of the light remain the same, the specimens, after becoming positive, remain so all the rest of the day, or (if exposed to strong sunlight) until overcome by the heat.

The fact that many specimens after being taken from under the seaweed are, for a short time, negative, suggested the view that the animals are rendered negative by being kept in darkness. Under the seaweed some specimens may not be exposed to light at all, and others exposed in varying degrees, — circumstances which may account, in great measure, for the varying reactions of these animals when first captured.

To test this conclusion a large number of specimens was collected on the beach and divided into two lots. At first the specimens were about equally positive and negative. One lot was exposed to the light in a room lighted by a north window, the other lot was placed in the dark. After a little over five hours those that were kept in the dark were removed. All of the specimens were strongly negative, while those that were exposed to the light were almost all positive. The individuals that were kept in the dark were then exposed to

direct sunlight where they remained negative for a short time; but some soon began to wander to the positive end of the dish, and in fifteen minutes more than two-thirds had become positive.

Hence it would seem that darkness causes Orchestia to become negative for a short time after exposure to light.

Orchestia palustris. — Orchestia palustris is much less affected by light than the two preceding species. If a number of specimens are exposed to the light they do not all rush toward it; nevertheless several will show a marked positive phototaxis. More individuals that are negative are met with under ordinary conditions than in Orchestia agilis, and the negative condition persists longer. Owing to the somewhat dilatory manner in which the specimens react to light, few observations were made on this species. The weak responsiveness of palustris to light may be correlated with its habits of life. It is found around marshes under grass or weeds on the damp ground. While not exposed to bright light it does not live in such dark situations as Orchestia agilis or, still less, Talorchestia longicornis, and is perhaps less sensitive to photic stimuli on this account. In the three species described positive phototaxis becomes less decided in proportion as the amount of light to which they are habitually exposed is increased.

Orchestia agilis rendered Negatively Phototactic by being transferred from Strong to Weak Light.

The experiment of bringing Orchestias from strong to weak light gave a remarkable and unexpected result. If specimens that show a marked positive phototaxis in direct sunlight — as all do if they remain in it for some time — be brought into a much weaker light they soon become to a marked degree negatively phototactic. This unlooked-for result caused me to try the experiment repeatedly and under different conditions. Accounts of a few experiments are here given:

On August 31, a large number of Orchestias was collected on the beach at 4 P. M. When first taken they were mostly positive. They were at once brought into the laboratory and exposed to the weaker light of a north window. Almost all of the specimens quickly became negative. In the course of half an hour they began to move more and more to the positive end of the dish, and at 4.45 almost all were at that end. At 7.30 P. M. (in the lamplight) all, several hundred in number, were positive.

On September 1. at 8.30 A.M. numerous Orchestias were collected, which were at first almost equally positive and negative. After twenty minutes' exposure to sunlight almost all had become positive. They were then taken into a small dark-room with a small window where they quickly became negative, with the exception of about half a dozen individuals out of several hundred. After exposure to light in this room for an hour about half the specimens became positive. They were then exposed, surrounded by ice, to direct sunlight, in which practically all became positive very quickly. Experiments essentially like the above were repeated several times.

It was found that increasing the temperature, at least under certain conditions, renders Orchestia agilis more strongly positive. In bringing specimens from direct sunlight to a dimly lighted room differences of temperature are encountered which might be considered to effect the change in the sense of phototaxis. If specimens that are negative in a dimly lighted room be brought into direct sunlight and there become positive this change might be attributed to the increased temperature and not to the difference in the intensity of light; and when the specimens are brought back into the dimly lighted room and become negative again, it might be thought that their natural negative phototaxis had reasserted itself as the temperature fell. The inadequacy of this interpretation is best shown by the following experiments:

A lot of Orchestias was collected at 11 A.M. At first, in direct sunlight, more than nine-tenths were positive. When brought into a dimly lighted room they became strongly negative, the temperature in the dish being 78° Fahr. They were then placed in a dish surrounded by cold water and exposed to the sunlight. Although the temperature was a few degrees lower than in the dimly lighted room the majority of specimens quickly became positive.

A second lot was placed in a dish surrounded by ice water and exposed to the direct sunlight, the thermometer in the dish registering 72° Fahr. All of the numerous specimens were positive except a very few. When the dish was taken to a dimly lighted room where the temperature became 74° Fahr. all the specimens became negative. When the dish was turned all the specimens went to the negative end of the dish again. The dish was then quickly heated to 84° Fahr. All but two or three of the specimens were still negative. The dish was turned and all but four or five (out of about one hundred) went to the negative end again. The dish was then put back into the ice water and exposed to direct sunlight where the temperature ran down several degrees. The specimens quickly became positive and rushed back to the positive end again when the dish was turned. On returning the dish to the dimly lighted room where the thermometer again showed a rise the specimens again became

strongly negative, going back to the negative end each time the dish was turned. The dish was then exposed to direct sunlight without being surrounded by ice water and the specimens became positive, the thermometer rising to 94° Fahr.

Thus, when specimens are brought from strong to dim light, they are made negatively phototactic whether this change be accompanied by an increase or a decrease of temperature; and, conversely, when specimens are transferred from weak to strong light, they are made positively phototactic whether they be brought into a colder or a warmer temperature than before. Differences of temperature, therefore, cannot account for the changes thus produced in the sense of phototaxis.

Where change in the sense of phototaxis is due to the influence of light itself it has been found hitherto that strong light tends to produce negative phototaxis. Many forms, like the swarm spores of algæ, and the larvæ of Polygordius, are positive in light up to a certain degree of intensity, their optimum, beyond which they quickly become negative. It may be necessary to distinguish two ways in which the response of an organism may be modified by light, the direct effect of changed intensity of stimulation, and the indirect effect of previous exposure. When a swarm spore, previously positive, turns about immediately upon an increase in the intensity of light, the change of reaction is apparently the direct effect of increased stimulation. The effect of previous exposure is shown by many swarm spores, as has been demonstrated by Strasburger.[1] Groom and Loeb[2] found that Balanus larvæ were positive in the morning, even in the sunlight, but gradually became negative during the day. If after the larvæ have become negative, they are kept in the dark for a short time and then exposed to the light, they become for a short time positive again. Light of the same intensity makes the larvæ positive or negative, depending upon the intensity of light to which they have been exposed. This is illustrated also by the fact that continued exposure to strong light makes some forms positive in light in which otherwise they would have been negative. The effect of bringing Orchestias from strong to weak light, as well as the effect of keeping them in darkness, is quite different from that which has been found in other organisms, and it is difficult, there-

[1] Strasburger: Jenaische Zeitschrift, 1878, xii, p. 574.

[2] Groom and Loeb: Biologisches Centralblatt, 1890, x, p. 166.

fore, to bring all the facts under a common explanation. The fact that bringing Orchestias from strong to weak light changes their phototaxis from positive to negative, is difficult to reconcile with the doctrine often assumed that negative phototaxis is due to stimulation above the point of optimum intensity. Perhaps this explanation may be true for some cases of negative phototaxis, though not for all.

Change of Phototaxis produced by throwing Terrestrial Amphipods into Water.

As it seems to be a general rule that the terrestrial amphipods are positively phototactic, while the aquatic species are negative, it occurred to me to determine if any effect on phototaxis would be produced by placing terrestrial amphipods in water. It was found that if strongly positive specimens of Orchestia agilis are placed in water, they immediately became strongly negative. This change is very marked; specimens that jump furiously toward the light immediately swim as vigorously away from the light when they are placed in water. The negative phototaxis of these animals in the water seems to be independent of the intensity of the light; they are strongly negative in direct sunlight and in light of very weak intensity. Specimens were transferred from direct sunlight to dim light and back again; but they remained negative throughout. And it is not merely the majority of individuals that become negative. If several hundred are placed in a dish of sea-water they will usually, without a single exception, proceed to the negative end of the dish. I have never observed one of this species that could be regarded as positively phototactic when first placed in sea-water. If the specimens are removed from the water, they quickly become positive again. I have repeatedly changed specimens in rapid succession from positive to negative and the reverse, by placing them in water and then taking them out. It was shown that differences in temperature do not produce this change. Specimens were placed, some in water of the same temperature as the air, some in water colder, and others in water warmer than the air; but in all cases the animals previously positive became strongly negative. A wide range of temperature was tried, from water much colder than the air to water of 90° Fahr., without producing a change in the sense of phototaxis. When the point where heat rigor supervenes is approached, the

animals tend to become positive, as will be described further on. There can be no question, however, that differences of temperature do not account for the change of phototaxis that occurs when the animals are thrown into water.

Water of different degrees of salinity was tried. In ordinary sea-water specimens were observed to remain negative for several days. How long the animals will live in sea-water is unknown, but those kept under observation remained negative as long as they were kept in it. In concentrated sea-water specimens remained negative for several hours, — as long as life was retained. Specimens placed in perfectly fresh water likewise became strongly negative, but they lived in this medium only for a few hours. For a short time before they died in the fresh water, however, they showed a tendency to become positive.

On August 21, at 10 A. M., I collected several hundred specimens, allowing them to jump directly into a dish of sea-water, where all immediately became negative. The lot was taken into the laboratory and divided into three parts, one portion being placed in fresh water, another portion placed in a dry dish, and the remaining ones left in the sea-water. The specimens in the dry dish were at first mostly negative, but gradually became positive. At 12 o'clock the specimens in fresh-water, like those in the sea-water, were all negative. At 4 P. M. the fresh-water specimens were more positive than negative. At 5 P. M. they were almost all positive, while those in the sea-water were all negative, and the same condition obtained at 6 P. M. At 8 P. M. the specimens in fresh water were markedly positive, while those in the sea-water were negative. At 9.30 the same condition obtained. At 8.30 the next morning the specimens in sea-water were still negative, while those in the fresh water remained positive. Later in the forenoon the specimens in fresh water died. Essentially the same experiment was tried several times, with similar results, the specimens in fresh water after several hours becoming positive and afterwards dying.

The behavior of Talorchestia longicornis, when placed in water, is different from that of Orchestia agilis. Talorchestia while in the air is positive under all circumstances in which I have seen it placed. When thrown into sea-water it shows a comparatively weak tendency to go away from the light, but this is only temporary, as in the course of an hour or more the animals become positive. The movements of Talorchestia in the water are much less vigorous than those of Orchestia. The animals seem dazed or stupefied, especially during the first hour, and often float passively for some time without

making much of an attempt to swim. I give here the behavior of one lot of Talorchestia as it is recorded in my notes: —

August 28. At 11.02 A. M. eight specimens of Talorchestia were placed in an oblong dish of sea-water at 72° Fahr. At 11.04 they had all assembled at the negative end of the dish, where they moved about more or less in the endeavor to get away from the light. At 11.05 one went to the + end, but returned at 11.15. They all remained at the — end until 11.44, when one specimen went to the + end. Another specimen went to the + end at 11.45, but returned at 11.46. The specimen that went to the + end at 11.44 returned at 11.48. At 11.46 another went to the + end, but immediately returned. All remained at the — end until 12.10, when one went to the + end, and at 12.13 another followed, one returning at 12.14. Two were at the + end at 12.17, and but one at 12.28. Then several began to move into the central part of the dish, apparently indifferent to the light. (Until this time the specimens kept to the one end or the other of the dish.) At 1.43, seven were at the + end of the dish and one at the — end. At 1.57 the negative specimen came to the + end, and all remained at that end until 2.08, when the observation was interrupted. When observed at 3.10 and at 3.50, all but two were at the + end. At 3.50 all the specimens were carefully placed at the — end. Three immediately swam to the + end, and in less than a minute five were at that end, and in four minutes all were there. The dish was then turned about; all but one came to the + end in less than a minute, and that one in less than two minutes. At 3.58 the dish was again reversed; all the specimens went to the + end in less than a minute. Then all were removed to the — end, and in less than a minute all were at the + end.

The experiment was tried again with the same result.

The experiment was tried again with the same result in less than two minutes, and again with the same result in less than one minute.

At 7 P. M., when again observed, all the specimens were at the + end. They were all removed at 7.40 P. M. to the — end, but returned to the + end in less than a minute. The experiment was then repeated three times with the same result. On August 21, three of the eight specimens were still alive after having been kept in water three days, and these were all positive, as they were on the day before.

The first reaction of the specimens in this experiment was negative. Then individuals seemed to become temporarily positive. Later, the tendency to go to the positive end of the dish began to predominate, and finally it became very decided. I have several times kept specimens until they ran through the changes from negative to positive. The initial negative reaction is not so marked as the

final positive response, and sometimes specimens when placed in the water seem so stupefied that they do not react in a definite manner either way. Later, however, such specimens almost invariably become markedly positive. The comparatively weak and temporary negative phototaxis of Talorchestia when placed in sea water is of interest when compared with the strong and apparently permanent negative reaction of Orchestia agilis under the same conditions. This difference is not improbably due to the fact that in the air Talorchestia is more strongly and unalterably positive. Contact with water which changes the positive phototaxis of Orchestia agilis produces but a relatively slight and temporary effect on the stronger positive phototaxis of Talorchestia.

It was found by Loeb[1] that in the young larvæ of Limulus the direction of the phototactic movements depends upon whether the animal moves by crawling or swimming. In the Amphipods, however, the direction of movement stands in no relation to their mode of locomotion. I have seen Talorchestias in the water crawl as well as swim both to and from the light, and Orchestia, when made positive by fresh water or excessive heat, swims towards the light just as, under ordinary circumstances, it swims away from it.

The cause of this curious change in phototaxis when the terrestrial amphipods are thrown into water is not apparent. Differences of illumination and of temperature certainly cannot account for it. It is possible that it may be correlated with differences in facility of respiration in the two media, but in this case we should not expect the change to be so brief in Talorchestia. The change appears to take place as soon as the animals are thrown into water. If it were due to changes in respiration it would be expected that a few seconds at least would be required to effect the transformation. It does not seem improbable that we have here a case in which the response to light is modified by thigmotaxis. Miss Towle[2] has shown that in Cypridopsis the sense of response to light may be changed by contact. Striking the side of the vessel, or any solid object, often changed the response from negative to positive. When a specimen was picked up by a pipette and dropped into the water "in almost all cases the first response was positive; and, where doubtful at first, it became steadily positive after the animal had been several times

[1] Loeb: Archiv für die gesammte Physiologie, 1893, liv, p. 80.

[2] Miss Towle: This journal, 1900, iii, p. 352.

disturbed." Similar results were later obtained by Yerkes in Cypris.[1] That the larvæ of Limulus are positive while swimming and negative when crawling on the bottom of the dish may possibly be also due to the effect of contact stimuli. When a terrestrial amphipod is thrown into water it immediately receives contact stimuli on all parts of the body. If contact stimuli produce a temporary change of phototaxis in Cypridopsis and Cypris, it is not unreasonable to suppose that when such stimuli act constantly a change of longer duration may be produced. This is merely offered as a suggestion, and I hope by further experiments to throw more light upon the problem.

The Effect of Temperature on Phototaxis.

Attempts to change the sense of phototactic movements in Orchestia by raising or lowering the temperature under the same conditions of light were unsuccessful so long as the specimens were kept in the air. As Orchestia can be made temporarily negative by being brought from strong to weak light there is an opportunity afforded for studying the effect of temperature on the rate of change from negative back to positive phototaxis. Experiments were conducted in the following way: A lot of Orchestias was exposed to strong light until the specimens became strongly positive. They were then taken into a dimly lighted room where they became negative and divided into two lots. Both lots were kept exposed to the same conditions of light. The dish containing one lot was warmed, — light from the source of heat being excluded, — while the other dish was left at the ordinary temperature. It was found that the specimens in the warmed dish became positive more quickly than those in the cooler one. As all the specimens had the same exposure to light before being taken into the dimly lighted room, and were exposed to the same intensity of light while one lot was heated, it is fair to conclude that increase of temperature accelerated the positive response.

As Orchestia agilis becomes negative when placed in water, experiments were tried in order to ascertain if increasing the temperature of the water would make the species positive.

Numerous Orchestias were placed in sea-water which was gradually heated. At 90° Fahr. the specimens were still negative. At 92° Fahr. two or three were apparently positive. At 100° Fahr. many were rendered inert by the

[1] Yerkes: This journal, 1900, iv, p. 416.

heat; but those that responded to the light were about equally divided between positive and negative. At 105° Fahr. almost all that could swim went to the positive end of the dish. Numerous fresh specimens were then dropped into the dish and they immediately swam towards the light, a few succumbing to the heat before reaching the end of the dish. After a few minutes not a specimen was at the negative end of the dish and several were swimming at the positive end in the endeavor to go towards the light. Soon most of the specimens were dead. The warm water was then poured off and replaced by cool sea-water. The individuals that were able to swim immediately went to the negative end of the dish and several that were apparently dead gradually revived and, when they became sufficiently vigorous, swam also to the same end.

This and a few other similar experiments indicate that increase of temperature tends to make Orchestia agilis positively phototactic, although the point where the transformation occurs lies very near the limits of the animals' power to withstand heat.

The effect of increasing the temperature was tried upon several aquatic amphipods, but generally death resulted before a change in the sense of phototaxis could be produced. Most of the species were unable to withstand a temperature of 100° Fahr. for even a few minutes. In some cases positive phototaxis was apparently produced shortly before heat rigor set in, but the results in general were too doubtful to be relied upon.

A specimen of Gammarus mucronatus, however, was found to endure easily a temperature of 100° Fahr. in which it became strongly positive. The dish in which the animal was placed was turned repeatedly and the creature swam quickly to the positive end each time. The response was very decided, but unfortunately no other specimen of this species could be obtained. When placed in cooler water this specimen became quickly negative, as before. The next day experiments with the same individual were repeated. It became markedly positive when placed in sea-water at 100° Fahr., and negative again when placed in cooler water as on the day before.

The influence of temperature on phototaxis differs in different forms. Strasburger [1] found that the swarm spores of Hæmatococcus which were positive at a temperature of 18° C. became negative if the temperature was lowered to 4° C., while, if the temperature was raised to 35° C., the positive phototaxis became more marked than before. In general, among the swarm spores, raising the temperature

[1] Strasburger: Jenaische Zeitschrift der medicinisch-naturwissenschaftlichen Gesellschaft, 1878, xii, p. 605.

was found to produce positive phototaxis, while lowering the temperature had the reverse effect. Massart[1] found that Chromulina was positive at 2° C., but negative at 5° C. On the other hand, in the larvæ of Polygordius and some of the Copepods Lœb[2] found that raising the temperature produced negative phototaxis, while lowering the temperature made the animals more positive. The different effect of temperature on the reactions of different forms is only one of the many paradoxes that the subject of phototaxis presents.

Reversal of Phototaxis in Jassa produced by Foul Sea-water.

Upon observing some specimens of Jassa that were kept in a dish in which the water was allowed to become foul I noticed that the animals manifested a strong proclivity to swim towards the light. If the dish containing the specimens was turned, most of the specimens would return quickly to the positive end. Several specimens were then transferred to a dish containing fresh sea-water. Here they quickly became negative, like other specimens of this species that had been kept in sea-water that did not become foul. Both dishes were exposed to light of the same intensity. On being again transferred from the fresh to the foul sea-water the specimens again became positive. Several other species of aquatic amphipods were then placed in the foul water, but all of them retained their usual negative reaction. No investigation was made of the cause of the change in the sense of phototaxis in this species. The fact of change of phototaxis produced in this way seems, however, worthy of record.

The Effect of Blackening over One Eye.

While observing the negative phototaxis of a small fresh-water amphipod Hyalella dentata (Smith), it occurred to me that, since orientation in the direction of the rays of light is presumably determined by rays of light falling unequally on the two sides of the animal, if one eye were blackened over, shutting out photic stimulation from that side of the body, the animal would possibly perform circus movements in one direction. In several specimens of Hyalella one eye was accordingly blackened over with asphalt varnish, and it was found that the animals swam about in circles with the unblackened eye

[1] Massart: Bulletin de l'académie royale des sciences, des lettres et des beaux-arts de Belgique, 1891, xxii, p. 164.

[2] Loeb: Archiv für die gesammte Physiologie, 1893, liv, p. 91.

looking away from the centre of the circle. If the right eye is blackened over the animal veers continually around to the right, or if the left eye is blackened over it turns toward the left. Being desirous of trying the same experiment on an animal that is positively phototactic I tried first blackening over the eye of the common blue-bottle fly. In this case it was found that circus movements are produced, but that they occur in a direction opposite to those of Hyalella. If the right eye of the fly is blackened over the insect travels in circles to the left, or if the left eye is blackened it turns toward the right. The same experiment was tried on several positively phototactic species of insect, viz., two species of bees, the robber fly, Asilus, Tabinis, and a species of syrphid, and it was found, in each case, that the insects performed circus movements in a direction such that the unblackened eye looked away from the centre of the circle. The terrestial amphipods Talorchestia longicornis and Orchestia agilis show, when one eye is blackened over, a tendency to turn in the same direction which is very marked. On one occasion, when returning to my room, I observed a specimen of Talorchestia with one of its eyes blackened that had escaped from its box and was busily engaged in trudging around on the floor in a circle about six inches in diameter. It kept up its circular movements in the same path for several minutes, when it finally stopped, apparently through fatigue. As Orchestia agilis is positive or negative depending upon whether it is in air or water it can be made, when one eye is blackened over, to perform circus movements in the one or the other direction by changing it from one medium to the other. Blackening over the eyes of several aquatic amphipods caused these animals to perform circus movements in the same direction as Hyalella dentata. Destroying one eye in Talorchestia was found to produce the same circus movements as blackening over the same. When both eyes are destroyed phototaxis no longer appears.

Thus far my experiments have borne out the rule that, in positively phototactic animals, blackening over one eye causes circus movements to be performed with the unblackened eye looking away from the centre of the circle, while in animals that are negatively phototactic the same operation causes circus movements to be performed in the opposite direction.

The Effect of Hemisecting the Brain.

When a terrestrial amphipod moves so as to face the source of light the legs on the side away from the light act more vigorously than the others, thus turning the animal so that its long axis becomes parallel with the rays. If this turning is a reflex response to stimulation by light we must suppose that impulses generated in the eye turned toward the source of light are conveyed to the appendages on the opposite side of the body. What is known of the nervous system in other crustacea renders it very probable that in the amphipods a large proportion of fibres from the optic nerves cross in the brain. When the brain, therefore, is cut through the middle one chief route by which impulses are conveyed from the eyes to the opposite side of the body is destroyed. It has been found by Bethe,[1] in Carcinus and Astacus, and also in the water-beetle Hydrophilus, that, when the brain is cut through in the middle, the animals are no longer negatively phototactic, although their other actions soon come to be very much like those of normal individuals. As it was a matter of interest to determine the effect of hemisecting the brain in forms with such strong positive phototaxis as the terrestrial amphipods, Talorchestia was chosen for this experiment, as its large size renders it a favorable form for the purpose. The brain was cut in two with a sharp, finely pointed knife, the cut being made deep enough to leave no chance that the separation of the two halves of the brain should be incomplete. Owing to the profuse bleeding thus caused many specimens die, but quite a number recover and may be kept for several days in a dish of moist sand. One specimen thus operated upon was kept for two weeks. The specimens soon behave in a normal manner, burrowing in the sand and often coming out of their holes at night. While their behavior shows that they are affected by the light, phototaxis seems to be entirely destroyed. In no case could I detect the least power of orientation to light. The same experiment was tried on a few positively phototactic species of insects, — Smerinthus, Junonia, the honey-bee, and the blue-bottle fly. These forms were kept alive for two days after the operation, but showed no trace of phototaxis, although they were irritable to light. This loss of phototaxis is, I believe, not entirely the effect of the shock of operation, or of incidental injury to other paths of photic impulses, although the

[1] Bethe: Archiv für die gesammte Physiologie, 1897, lxviii, p. 449.

experiments are not conclusive on this point. Delle Valle found that when he destroyed, or very much mutilated, one half of the brain in Orchestia, the animals, after a few gyrations, would jump towards the window in their usual vigorous manner. This makes it rather more probable that the loss of phototaxis in Talorchestia is due not to the rough treatment of the nervous system, but to the fact that the nervous connections necessary for the phototactic response have been severed. We are not yet in a position to come to positive conclusions on this matter.

Phototaxis and Photopathy.

There are commonly distinguished two modes by which animals react to light, — phototaxis, in which the body orients itself to the direction of the rays, and photopathy, in which individuals collect in an area of a certain intensity independently of orientation. It was formerly held that the phototactic movements of organisms were due to a tendency to seek a certain intensity of light. It has been shown, however, by Cohn and Strasburger for the swarm spores of algæ and some flagellates, and by Loeb for several animals, that it is the direction of the rays and not differences of illumination in different areas that causes the direction of movement. Positively phototactic forms move toward the source of light even if brought thereby into an area of less illumination, and negatively phototactic forms move away from the source of light even if they are thus brought into an area of greater illumination. The direction of the rays, therefore, has been emphasized as the important factor in phototaxis. It was pointed out by Loeb[1] that certain animals show a sensitiveness to changes in intensity of light without being noticeably oriented by the rays. An example of this, as Loeb held, was afforded by the fresh water planarian, Planaria maculata. When specimens of this species were placed in a round dish before a window they collected at the sides of the dish where it was shaded, and not at the point farthest from the window as do forms which move away from the light in the direction of the rays. This was explained by Loeb on the ground that, as the planarians are stimulated to activity by light, they come to rest only when they accidentally get into a shaded area, where they would thus naturally form collections. Such a mode of response to light Loeb distinguished from phototaxis (or heliotropism) as Unterschieds-

[1] Loeb: Archiv für die gesammte Physiologie, 1893, liv, p. 101.

empfindlichkeit, or sensitiveness to changes of light intensity, and several later writers have concurred in this distinction.

In an article on "The Theory of Phototactic Response," in a recent number of this journal, Holt and Lee[1] have maintained that "the assumption is unnecessary and misleading, that the motor reactions of organisms to stimulation by light are of two kinds, namely, reactions to the intensity of light, and reactions to the direction of its rays. The phenomena that have led to such an assumption can be satisfactorily explained on the simpler theory that every ray of light impinging on an organism stimulates at the point on which it falls and in proportion to its intensity, and that, as a result of this, organisms always endeavor to seek their optimal intensity of illumination.

"The direction of the rays has in itself no effect whatsoever on the movements of the organism. It is true, however, that if the rays reach the animal from a certain side, that side of the body is stimulated more than the other, for the other side lies in its own shade. Hence the controlling response is called out on the first side; but exactly the same response would be produced if the same side of the organism could be similarly stimulated by rays coming from any direction whatsoever. The direction of the rays is therefore a secondary factor, operative only in so far as it determines what side of the organism shall be stimulated. . . . By means of these two factors alone, the intensity of the light, and the side of the organism that the light reaches, this paper has aimed to explain all the facts of the phototactic response. . . . The facts do not show that the direction of the rays is otherwise effective than in determining on what part of the animal the light shall fall. There is no evidence that the organisms respond to any other property of light than its intensity, and the distinction commonly made between phototaxis and photopathy as different forms of irritability is unwarranted."

The authors of this paper have, I believe, a somewhat exaggerated idea of the prevalence of the view that phototaxis is due to the direction of the rays *per se*. They treat as a "widely-accepted assumption" a position which, so far as I am aware, no writer on phototaxis in animals has ever seriously adopted. Loeb apparently leaves the possibility open that the direction of the rays may operate independently of intensity, although his statement of his theory implies that different intensities of illumination on the two sides of the body is an important factor in bringing about orientation. An animal oriented to

[1] Holt and Lee: This journal, 1901, iv, p. 479.

the rays moves neither to the right nor the left because "symmetrical points receive light of *like intensity at a like angle*." Davenport[1] has put forward a theory of orientation essentially like that of Holt and Lee, although he makes the alternative suggestion that the course the rays take through the organism may also be a factor in the case. This suggestion Holt and Lee oppose. They seem to think it represents Davenport's general theory of the phototactic response. The hypothesis that orientation to the direction of the rays is brought about by unequal intensities of stimulation on the two sides of the body affords a very simple and natural way of accounting for the phenomenon. Whether, in addition to the effect of light on the surface, the direction in which light enters the tissues may not have something to do with orientation, at least in some forms, we are not, I believe, in a position to decide. I think I am safe in saying that we have had no experiments in which the effects of direction of rays and of different intensity of light on the two sides of an organism have been separated.

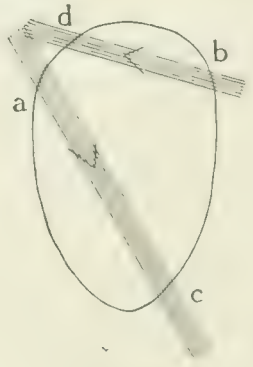


FIGURE 1.

The attempt might be carried out more successfully in plants than in animals. Until such experiments have been performed we are not justified, I believe, in asserting positively that the statement that light acts by the course the rays take through the organism is "erroneous." In a semi-transparent animal (Fig. 1), suppose that the two symmetrical points *a* and *b* are stimulated with equal intensity by rays of light coming in different directions. In the one case light travels through the animal in the direction *a c*, in the other in the direction *b d*. According to Holt and Lee, the direction in which light falls on *a* and *b* is immaterial, but it seems not improbable, *a priori*, that the ray *a c* traversing as it does a different part of the body from the ray *b d* would produce a different motor effect. The conditions are here

[1] DAVENPORT: Experimental morphology, 1897, i, p. 209.

fulfilled, so far as Holt and Lee's theory is concerned, for movement straight forward. Would the animal tend to move in this direction? We do not know; neither the experiments of Holt and Lee, nor any others that have been performed, put us in a position to decide.

If animals were perfectly transparent so that all parts of the body could be equally illuminated by parallel rays of light passing through it, the direction of the rays, according to this theory, could not produce any orientation. There would be nothing but pure direction of light to produce a change of axial relations. No organism fulfils this condition with respect to light, but all are perfectly transparent to the force of gravity, which acts with equal intensity on all parts, whatever may be their position. Yet this force orients organisms in a very marked way owing solely to the direction in which it acts. The analogy of heliotropic to geotropic effects was one of the main considerations that led Sachs to go farther than those who have studied phototaxis in animals and insist that direction of rays, *per se*, independently of intensity of illumination, is the most important factor in producing orientation. The course which an electrical current takes through an organism certainly plays an important part in electrotaxis independently of the intensity of stimulation at the points where the current enters and leaves the body. That light may act in a similar manner in forms that are semi-transparent does not seem improbable, much less "wholly mystical," as it appears to Verworn. While it may be possible that the direction in which rays pass through an organism is a minor factor, if a factor at all, in producing orientation, yet, since we have had no conclusive experiments on the subject, a positive conclusion is, I believe, at present premature.

Having resolved the responses of organisms to light into reactions to intensity Holt and Lee conclude that they have done away with "the distinction commonly made between phototaxis and photopathy as independent forms of irritability." Granting that their theory of orientation is correct, it does not follow that phototaxis and photopathy are not different forms of behavior towards light, whether or not we choose to call them different forms of irritability. There may be forms which are not oriented to light at all, but which, when in the light, are stimulated to move faster than when in the dark. Such forms would naturally collect in dark places independently of differences of illumination on different parts of the body. Or if forms are more active both in bright light and in the dark than in light of moderate intensity they would naturally collect, independently of any

orientation, in places of moderate illumination. This mode of reaction to light it is convenient to distinguish from phototaxis, and the term photopathy is applied to animals supposed to respond in this manner. Do both kinds of reaction occur in nature? There are many animals which show no apparent orientation to light, but which move from brighter into darker places. There may be in these forms an element of orientation not easily detected, but if it exists it does not wholly explain why the animals congregate in the dark. Parker and Burnett[1] have recently shown by statistical observations that Planaria gonocephala is to a certain extent oriented by the rays of light. Yet as planarians move about more rapidly in the light than in the dark they would doubtless collect in shaded places independently of their negative phototaxis. Phototaxis and photopathy are doubtless often associated in the same animal, their relative potency varying greatly in different species. Negative photopathy may be associated not only with negative, but also with positive phototaxis; this is shown by the fact that terrestrial amphipods, although having a strong tendency to go towards the light, habitually come to rest in shaded spots. The swarm spores of many algæ, on the other hand, swim about actively and continuously in the dark, and come to rest only after exposure to light, a mode of behavior which, independently of the positive phototaxis which they often possess, would lead them to collect in lighted areas which they must reach if they undergo further development. In these swarm spores positive phototaxis occurs along with what might be considered a form of positive photopathy, and the same relation may be found to exist among other organisms. Of the photopathic responses of animals we have very little knowledge. That aggregations of organisms may be formed, through the agency of light independently of orientation cannot, I believe, be reasonably questioned. In case of many forms that collect in dark places we may be reasonably sure that orientation is not necessary if it exists. The cases in which organisms have been held, independently of the effect of the direction of the rays, to seek light of a certain mean intensity, as in experiments with forms under darkened prisms, are open to the criticism that the possibility of a phototactic effect coming into play was not excluded. Holt and Lee have plausibly explained several purported cases of this kind as due to phototaxis. A photopathic response of this kind is of course possible, perhaps

[1] Parker and Burnett: This journal, 1900, iv, p. 373.

not improbable, but cannot, I believe, be considered as proven. The modus operandi of the photopathic response has received little attention. Most cases of photopathy are not improbably due to the fact that organisms are less active in some areas than in others and therefore collect where they are the most quiet. Still other modes of forming aggregates are possible by means of stimulation by light, but we do not know to what extent they are realized. If such modes are found to occur the question will arise whether the term photopathy should be used to include them. Photopathy of the kind just described undoubtedly occurs and is a different form of response from phototaxis, although both reactions are often, if not generally, exhibited to a greater or less extent by the same organism.

Summary.

All the aquatic species of amphipods studied are negatively phototactic.

The three terrestrial amphipods studied are positively phototactic under ordinary conditions, the reaction being more decided in proportion as the species is less habitually exposed to light.

Talorchestia longicornis is strongly and permanently positive both in very weak and in very strong light; nevertheless it has a strong proclivity to come to rest in shaded spots.

After being kept in darkness Orchestia agilis becomes temporarily negative; it then becomes positive again more quickly the brighter the light, and remains positive in the strongest light.

Specimens of Orchestia agilis that are positive in strong light may be rendered temporarily negative by exposure to light of less intensity. This change is independent of differences of temperature.

Negative specimens of Orchestia agilis become positive more quickly if the temperature be raised.

When thrown into water Orchestia agilis quickly becomes negative. In sea-water this change is apparently permanent but in fresh water the animals become positive some time before they die. At a high temperature individuals in sea-water tend to become positive.

Talorchestia longicornis when thrown into water is at first weakly negative, but afterwards slowly becomes positive.

A species of Jassa was found to become positive when placed in foul sea-water.

Blackening over one eye of the terrestrial amphipods and in several

positively phototactic species of insects causes the animal to perform circus movements with the unblackened eye looking towards the centre of the circle. Blackening over one eye in negatively phototactic amphipods causes circus movements to be performed in the opposite direction.

In Talorchestia and in some insects, it was found that after hemisection of the brain positive phototaxis was destroyed.

AN IMPROVED METHOD OF PREPARING AND PRESERVING MEAT FOR USE IN METABOLISM EXPERIMENTS.

By WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

THE chemical problems in metabolism experiments are as difficult as they are numerous. Not only must the excreta be analyzed in detail, but, in work of the highest value, the composition of the food must also be definitely ascertained. Usually, the purely analytic labor involved in studies of this character is so great that important phases of the experiments have to be ignored or left for subsequent special investigation. Methods of the greatest simplicity, which are easily carried out in the shortest time and with the highest degree of accuracy, are naturally the first to be selected. Consequently, any improvements of acceptable methods, which increase their adaptability in any one of these particulars, are to be welcomed.

The process the author has lately been employing to prepare proteid food in bulk for experiments on dogs includes a few improvements which make it perfectly adapted to metabolism work, and which, besides, lessen considerably the analytic and mechanical labor involved.

In the method referred to, the fresh lean beef, after all loose fat and connective tissue has been removed, and tendonous layers excised, is put through a meat-chopper. The hash thus obtained is then divided into portions of convenient bulk, and each portion is enclosed in cheese-cloth bags, and submitted to increasing pressure[1] as long as bloody fluid accumulates. Three to four hours are usually sufficient for getting rid of all fluid that can be separated. The compressed masses thus obtained may be kept under moistened cloth to prevent the surfaces from drying during the pressing of the remaining portions. Too much hash in the press makes thorough removal of surplus fluid impossible. In preparing about 50 kilos of the meat,

[1] The ordinary "meat press," employed for various purposes, such as the preparation of tinctures from herbs, etc., serves very well.

the author has found it convenient to press 6 to 10 kilos at a time. The size of the press in use would, however, naturally determine the amount of the hash to be pressed at one time.

The compact cakes are next broken in a large dish, intimately mixed by thorough kneading, and then very small quantities, picked out here and there all through the mass, are transferred directly to capacious tubes, weighed and analyzed.[1] Thus far we have not had occasion to make other than nitrogen determinations in the meat prepared in this manner. Excellent results were obtained with 2 to 3 grams for each analysis, although larger quantities may readily be utilized, perhaps with even greater accuracy.

Simultaneously with the sampling of the hash for analysis it should be quickly rolled between the hands into balls weighing about 50 to 100 grams. These are dropped lightly into wide-mouthed bottles of a capacity sufficient to hold five or six of the balls. The latter are not to be pressed together, but ought to rest very lightly on each other. The bottles are then promptly sealed and placed in a cold-storage room, where the temperature is maintained at or below 0° C. The meat-balls quickly solidify, and in the frozen condition can, of course, be kept indefinitely. After the balls are frozen there is usually a very light and delicate film of frost on the inside walls of the bottle, in places, indicating naturally that only a very slight quantity of water leaves the meat during the interval before the frozen state is reached. Under these conditions there is never sufficient movement of fluid to result in the formation of ice at the bottom. If, however, the frozen condition is not reached within a few hours, and maintained, bloody fluid is certain to trickle slowly to the bottom, in spite of the preliminary removal by pressure, thus changing the composition of the substance throughout the entire mass.

The hash prepared and kept in this way retains its normal appearance, odor, and taste for a very long time. If the bottles are small, containing little more than enough for one, or at most two days' feeding, practically no change can take place while material is being withdrawn, if this be done quickly. The globular form is of particular

[1] If the tubes are weighed after they have been thoroughly dried at room temperature, and before the hash is put into them, any interior condensation of water from the meat would be included, as it should be, with the weight [by difference] of prepared substance. This procedure would serve very well for nearly all of the analyses commonly made. The hash should, of course, be completely removed from the weighing tube in each determination.

advantage, in this connection, because it makes the removal of the meat, even in the frozen condition, very easy. When it is desired to take out meat for use, the bottles need to be kept at room temperature for only a few minutes before the delicate icy connections between the balls have thawed sufficiently to permit of easy withdrawal. Special thawing of the contents in bulk, in order to take out a sufficient supply of meat, is unnecessary. The balls remaining after each removal may be speedily returned to the cold-room without undergoing any change to speak of. The weighing, after removal, may be made very accurate by shaving off sufficient from an additional ball to give the desired quantity.

After the weighed meat has been placed in the feeding-dish, the hash soon softens and is ready for ingestion in a few minutes. Its treatment after removal from the bottle must naturally depend upon the requirements of the experiment in which it is to be used. In the researches in this laboratory on dogs in nitrogenous equilibrium, the meat has been weighed in a common glass crystallization dish,[1] in which were also placed definite quantities of cracker dust and lard, with subsequent addition of given proportions of water. On thoroughly stirring this mixture, the balls quickly fall apart, and, if the quantity of water is not excessive, the fluid finally has the consistency of thick soup. The odor of fresh meat is predominant when the cracker dust and lard are not too great in amount. Gentle warming suffices to raise the mixture to the ordinary temperature. It may be added that dogs eat this mixture very readily for weeks. Further, it is very digestible and nutritious.

To answer the question whether any important changes in the chemical composition of the meat take place during prolonged periods of preservation, the nitrogen content was determined in two samples of each of several preparations, at intervals of about ten days, for several weeks, with the results tabulated below.[2]

The analytic data obtained not only show the general uniformity in composition of meat preserved in this way, but demonstrate, likewise, that no important chemical alteration takes place at any time

[1] In shape the common glass crystallization dish is very well adapted to the licking up of last portions. Because of its transparency the operator can also easily bring together to the centre the fine particles which the animal missed at first, thus favoring final ingestion of the entire meal.

[2] The analyses were made by the Kjeldahl method. The quantities of hash used varied from 2.1362 to 3.3550 grams.

during the period of preservation, if the proper precautions are observed. The unimportant fluctuations in nitrogen percentages in the table are all within the limits of unavoidable error of analysis. The average percentages emphasize the fact of perfect uniformity throughout.

Percentages of Nitrogen.

Preparation. No.	Before freezing.	After freezing.		
	At time of preparation.	10 days.	21 days.	30 days.
1	3.58 3.49	3.56 3.51	3.57 3.45	3.58 3.57
2	3.60 3.55	3.58 3.46	3.69 3.59	
3	3.58 3.67	3.60 3.59	3.64 3.58	3.59 3.67
4	3.69 3.73		3.70 3.75	3.64 3.68
Averages.				
1	3.53	3.53	3.51	3.57
2	3.57	3.52	3.64	
3	3.62	3.59	3.61	3.63
4	3.71		3.72	3.66

It may be suggested that the use of this method is impracticable where special cold-storage facilities are lacking. It can be said, however, in anticipation of such a conclusion, that practically the same satisfactory preservative results could be obtained, although with less convenience, of course, if the bottles were placed in an ordinary refrigerator and surrounded each day with the common freezing mixture of crushed ice and salt. Melting of the ice would not be very rapid, under these conditions, and it could be renewed at little expense whenever necessary.

The chief advantages gained by the use of nitrogenous food material prepared by the method just described are: —

1. The perfect freshness of the food at the time of its consumption, even weeks after its preparation; therefore, its similarity in appearance, odor, and taste to ordinary fresh meat, and its superiority to forms of nitrogenous food to which the animal is unaccustomed, or for which it has no desire.

2. The constancy of composition of the food throughout even the longest experiments, by which circumstance the labor of analysis is reduced to a minimum.

This method is therefore especially useful in metabolism experiments on dogs.

ERGOGRAPHIC STUDIES IN NEURO-MUSCULAR FATIGUE.

By THEODORE HOUGH.

[*From the Biological Laboratory of the Massachusetts Institute of Technology.*]

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Ergographic Methods.

THE observations recorded in this paper are the results of experiments which have been in progress since the summer of 1897. At first Lombard's modification of Mosso's ergograph was employed to measure fatigue. Changes were introduced into the apparatus from time to time until the ergograph used in all experiments described in the present paper differed in many important respects from the original instrument. Some of these changes, such as the substitution of the spring for the weight, have been independently introduced and described by others (Binet, Cattell); the final form of the instrument has not, however, so far as I am aware, been employed elsewhere, and consequently should be described in some detail.

The essential features of the instrument are shown in Fig. 1. The movement used was flexion of the middle finger at the joint between the first and second phalanges. The hand was secured firmly in the prone position to a wooden rest (shown in Fig. 2), by straps passing over the dorsal surface of the palm, the first and third fingers, and the first phalanx of the second finger. The thumb and fourth finger were left free. A V-shaped notch in the rest permitted flexion of the

second and third phalanges as described. Flexion took place against the resistance of a moderately strong spring, S, whose extension was directly recorded by a magnifying lever, L, upon the smoked surface of a revolving drum. The spring gave an extension of 2.5 mm. for each kilogramme of pull.

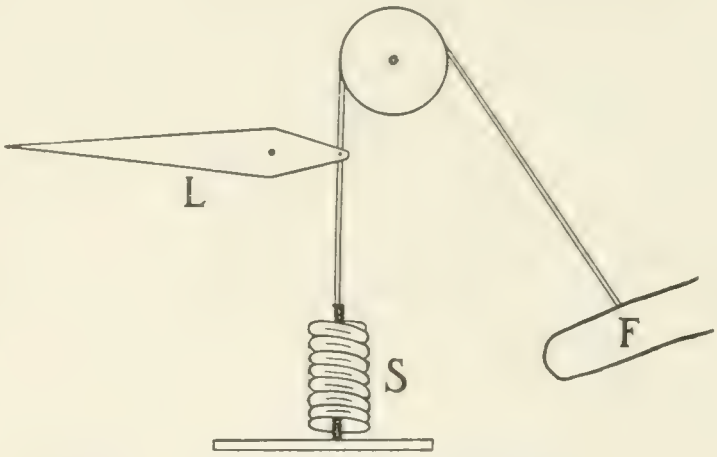


FIGURE 1. — Diagrammatic representation of the ergograph used in these experiments.

My procedure thus differs from that of Mosso in the following points: in the use of the spring instead of the weight; in the prone instead of the supine position of the hand and forearm; in confining the flexion to the second and third phalanges; and in the maintenance of a constant leverage for each subject of experiment, as will be described later.

The use of the spring instead of the weight. — It is unnecessary to explain at length the reasons for this change, as they have been given by Binet, Cattell, and Franz.[1] The spring will always give an approximate indication of the working capacity of a muscle, no matter how great the fatigue, whereas with the weight a point is usually reached sooner or later in which the muscle can no longer lift the given weight although still capable of lifting a smaller weight. Evidently the failure to shorten gives an incorrect record of the working capacity or state of fatigue; thus the graphic record of a series of rhythmic maximal contractions against the resistance of a spring becomes a more

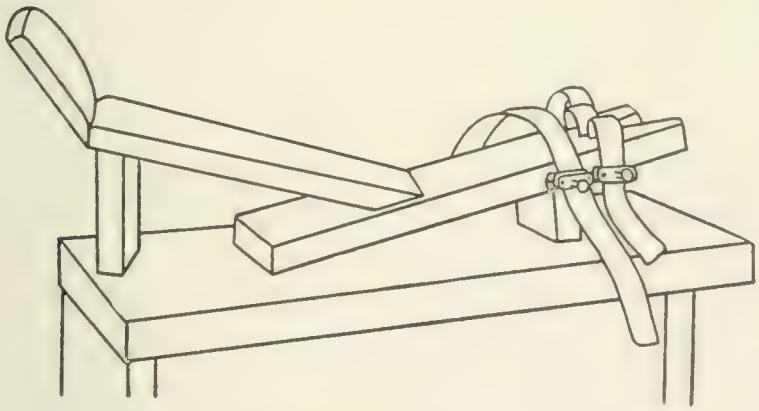


FIGURE 2. — Wooden rest for left arm and hand.

[1] FRANZ: This journal, 1900, iv, p. 349, gives the literature of this subject.

accurate curve of fatigue than that of a series of contractions with a constant weight.

It must at the same time be remembered that, in the study of fatigue, the use of the weight presents certain advantages over that of the spring. At the meeting of the American Physiological Society in New Haven, December, 1899, the writer pointed out that the work of our muscles consists for the greater part either in sustained contractions or tone, as in the work of the various antagonists engaged in maintaining the erect position, or else in a series of liftings of an approximately constant weight, and that this weight is usually far below the maximum for the given muscle. It is only necessary to recall walking, running, wood-chopping, rowing, swimming, etc., to realize that the weight ergograph actually represents the conditions which produce fatigue in the daily life of the human body; and it would be a serious mistake to abandon altogether this type of instrument in fatigue experiments.

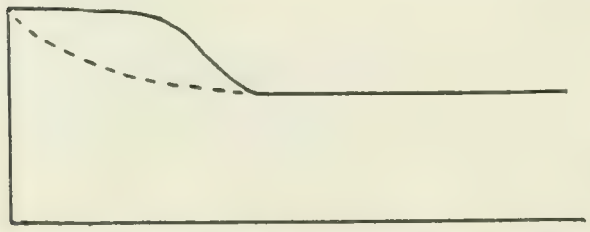


FIGURE 3. — Curves of fatigue with moderately heavy weight. The unbroken line represents the fatigue curve when the entire middle finger is flexed; the broken line, when flexion occurred only at the first interphalangeal joint.

The prone instead of the supine position. — This change, suggested by the description of Zimmermann's ergograph in the first volume of the "Intermédiaire de Biologistes," was adopted because the prone position is less constrained, and it is much easier to secure perfect fixation and immobility of the hand when the straps are passed over the dorsal side of the palm and fingers; the latter then flatten against the rest, while the strap readily accommodates itself to the curved, bony surface of the dorsal side.

Flexion at the first interphalangeal joint. — It has been the universal custom to flex all three phalanges. This procedure is, however, not permissible in studying the fatigue of a simple neuro-muscular mechanism,[1] because while the second and third phalanges are flexed by the mm. flexores digitorum in the forearm, the first phalanx is flexed chiefly by the mm. lumbricales in the palm of the hand. Maximal extension of the spring must in this case depend on the perfect

[1] *I. e.*, a single muscle or group of anatomically similar muscles with the innervating neurones of the anterior horn.

coördination in the working of two very different sets of muscles, thus introducing into the experiment the third factor of coördination over and above those of strength of stimulation and condition of the muscle as regards fatigue. My attention was first called to this matter by the character of fatigue curves obtained with the weight ergograph and moderately heavy weights. The unbroken line in Fig. 3 gives one of these curves. Suspecting that the exhaustion of the lumbrical muscles for the given weight was the cause of the first rapid fall, I made the first phalanx fast and then obtained curves approximately represented by the broken line.

Accurate regulation of the leverage at which the muscle works. — With the exception of the work of Franz,[1] little or no attention has been paid to the matter of attaching the resistance at a constant distance from the axis of movement in the joint, the attachment usually being effected by a thimble, or else by a leather or metal ring around the second phalanx. No special precautions have been taken to prevent the slipping of the ring or thimble, or to insure absolute uniformity of its position in different experiments on the same individual. And yet it can be easily shown that, as would be expected from consideration of the mechanical relations involved, a change of one or two millimetres in the point of attachment has a marked influence upon the curve of fatigue.[2] In order to avoid this error, I have used a double metal splint (Fig. 4)[3] which was attached to the two distal phalanges of the middle finger. Flexion was thus possible only at the first interphalangeal joint, as already described. The dorsal half of the splint carried an adjustable hook (for the attachment of the splint to the spring) which was made fast at a constant distance from the joint, determined upon the flexed finger by a T-square, after securing the splint. The muscle consequently worked at the same leverage in all experiments. The neglect of this mechanical principle is a serious source of error in most ergographic work.

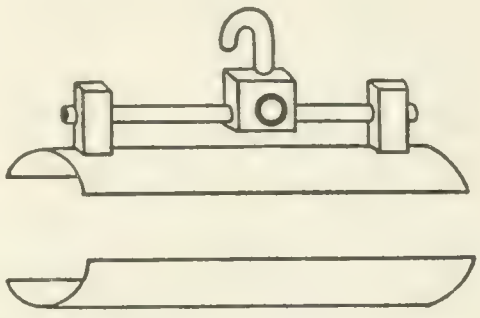


FIGURE 4. — The double splint used to attach the finger to the resistance.

[1] FRANZ: This journal, 1900, iv, p. 355.

[2] Compare the experiments of the first and second series in Table II on pp. 258, 259, and 260 of this paper.

[3] Described at the New Haven meeting of the American Physiological Society, December, 1899. See this journal, 1900, iii, p. ix.

It is very important that more attention be paid to the matter of leverage in ergographic experiments. It has been tacitly assumed that the pull of the flexor on the finger is comparable to the pull of an excised muscle on a weight which it carries. This is, however, by no means the case. In the first place, the attachment of the flexor muscle to the bone introduces a lever system, in virtue of which, as the bone passes from the condition of extreme extension to that of half flexion, the pull is applied at increasing mechanical advantage. In the second place, as the finger is flexed, the relative direction of its movement and of the application of the resistance constantly changes, so that, even could the weight be attached directly to the bone at the point of insertion of the muscle, the movement produced in the weight would be a constantly varying component of that produced in the bone. In the third place, the weight is not attached at the point of insertion of the muscle, but by a joint situated between one and two centimetres from this point; consequently, as the finger is flexed, of the total energy applied at this joint the component exerted in the direction of the pull of the weight varies. In Mosso's original ergograph, where the hand was placed in the supine position and the direction of the pull was that of complete extension of the finger, we have the very worst possible form of instrument in this respect, since the directions of application both of power and of resistance with reference to the direction of movement are at first those of the poorest mechanical advantage, improving as the finger is more and more flexed to the position of right angle. On the other hand, the arrangement described in this paper makes these two factors more or less successfully neutralize each other. Reference to Fig. 5 will show at once that, as the muscle pulls at increasing mechanical advantage, the energy of movement of the finger is applied to the resistance at decreasing mechanical advantage.

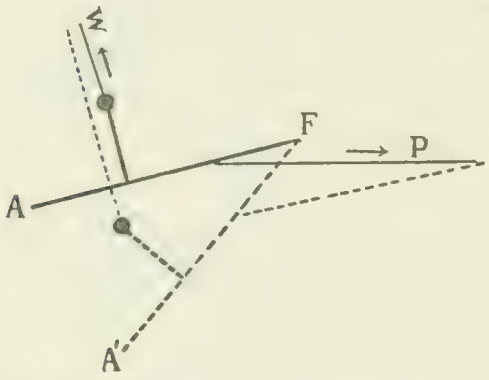


FIGURE 5. — Diagram showing changes in direction of application of the power (P) and the resistance of the spring (W) with increasing flexion of the finger. Initial position in solid lines; flexed position in broken lines. F = fulcrum of movement, F A = second and third phalanges of the middle finger.

One word with regard to the fixation of the forearm and hand.

As already described, the hand and forearm were placed in the prone position upon the wooden rest, notched at one end to permit downward flexion of the middle finger. The first and third fingers, the first phalanx of the middle finger, and the palm of the hand were strapped down as described (see Fig. 2). The forearm was not fastened in any way, since this is impossible without interference with the nutrition of the working muscle. Care was always taken, however, that the elbow should not be raised from the rest during contraction, and that the upper arm and shoulder should not move.

An objection has been urged against the use of the weight instrument, that the inertia of the weight carries the recording lever beyond the point of actual shortening of the muscle. Thus with the same weight and shortening the lever will give a higher record with a rapid than with a slow contraction. This is quite true and constitutes a valid objection, if the record is to be one of muscular shortening. It is not valid if the record is to be one of external work done, since the weight will in all cases be raised against the attraction of gravitation only so far as the muscle expends energy upon it; the higher record consequently represents greater external work on the part of the muscle. A muscle may shorten to the same extent when it lifts a ball in the hand as when it tosses the same ball into the air; the shortening in the two cases is the same; the external work very different; and it is the purpose of all ergographs to record the external work, not the shortening of the muscle.

It must be remembered that neither the weight nor the spring instrument records the total energy expended by a muscle; it is well known that, whenever a muscle contracts, a large part of the energy of chemical action leaves the muscle as heat, while the rest goes to mechanical work. The use of the spring undoubtedly gives us more accurate information as to the amount of chemical change taking place than does that of the weight; but we shall not have a perfect record of fatigue until we have some way of recording the total energy expended in each contraction, since all we know of the causation of fatigue shows us that this, and not simply the external work, determines the course of fatigue. Applying this to the "troublesome factor of inertia," we see that it is not a question as to whether the jerk or the slow contraction gives us the true record of the shortening, but which one represents more accurately the work done. Moreover, a spring can be stretched by the same two methods, thus raising a somewhat similar question as to the

more suitable method of contraction. I am not sure that we have at present the experimental data upon which to determine this question.

Before passing to the description of the results which it is the chief purpose of this paper to communicate, it may be well to call special attention to one unavoidable error, whose effect upon the tracing may, and generally is, mistaken for neuro-muscular fatigue. In order to secure a reliable record, it is, of course, necessary to fix the hand firmly to the ergograph and the splint to the finger; and it is impossible to do this without interfering with the circulation of blood in the palm and fingers. This interference may take the form either of venous congestion or of arterial anæmia. The splint, of course, can and should be fastened so as to avoid congestion in any part of the finger; but to do so inevitably involves the stoppage of the circulation, the chilling of the finger, and consequent unpleasant sensations, which so interfere, especially in the untrained, with the action of the will, that maximal contractions are not called forth. Gradually one becomes accustomed to these sensations, — indeed, becomes almost unconscious of them except in very prolonged experiments. It is doubtful, however, whether the afferent nervous impulses in question are ever without effect upon the strength of the efferent nervous stimulus, and so upon the height of contraction. In the other fingers and in the palm, on the other hand, congestion appears in ten or more minutes, and this similarly is not without its effect on the curve. It is evident that these effects must be constantly borne in mind in the interpretation of results, and especially in those from persons unaccustomed to the use of the instrument.

In the same way the subject must become used to the unavoidable tediousness of the experiment. All who have used the ergograph know how the seconds lengthen into minutes as the experiment proceeds. Gradually this feeling wears away, at least in a large number of subjects, so that a single experiment may easily last twenty or thirty minutes without undue tediousness.

The Fatigue of the Trained Muscle.

When an untrained muscle makes a series of contractions against a strong spring, a soreness frequently results, which cannot be regarded as a phenomenon of pure fatigue. In the trained muscle,

on the other hand, this complication is usually absent and the course of events is very regular and typical. It is consequently convenient to begin with the fatigue of the trained muscle.

The fatigue curve of a series of maximal rhythmic contractions. — We shall indicate the periods of work and rest by the convenient formula $\frac{C}{R} = \frac{m \text{ sec.}}{n \text{ sec.}}$, where C represents the period of contraction and R the period of relaxation and rest before the succeeding contraction.

Ergographic work has usually been done with the rhythm $\frac{C}{R} = \frac{1 \text{ sec.}}{1 \text{ sec.}}$ In most of my experiments I have used, instead, the rhythm $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.},}{\frac{3}{2} \text{ sec.},}$ the metronome beating half seconds. The latter rhythm is not so monotonous as the former and a trained muscle can reach its maximal contraction in a half second as well as in a second. We accordingly find that the height of the first contraction is not affected by the rhythm; we might, however, expect that since the period of rest is half as long again with the rhythm $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.}}{\frac{3}{2} \text{ sec.},}$ recovery between contractions would be greater, with a consequent diminution in the amount of fatigue. This, however, does not seem to be the case with regard to the two rhythms in question and with the one subject of experiment upon whom a number of such tests have been made. It should, however, be understood that this statement is not meant to be of general application: it is made only as bearing on the experiments given in the present paper.

Fig. 6 reproduces a tracing which may be regarded as perfectly typical of my results with trained muscles. The curve of fatigue falls as an asymptotic curve and ultimately establishes a practically constant level from which it does not subsequently change, except as the congestion or other unfavorable conditions in the constrained fingers or palm render subsequent work more difficult and the maintenance of the fatigue level impossible.

Tracings of this kind were exhibited at the meeting of the American Physiological Society already referred to, though they were not described in print until the publication of a preliminary abstract of my results.[1] Soon after this paper was sent to the printer, Schenck's paper, "Ueber die Verlauf der Muskelermüdung bei willkürlicher

[1] HOUGH: Journal of the Boston Society of Medical Sciences, 1900, v, pp. 81–92.

Erregung und bei isometrischen Contractionsact"[1] was received at our library. Working with the abductor indicis and with a modification of Fick's apparatus for the study of isometric contractions in man, it is gratifying to see that Schenck has found substantially the same form of fatigue curve as I have described, although using a different muscle and another form of apparatus.

While Schenck recognizes the establishment of a constant level as the striking characteristic of the fatigue curve for a skeletal muscle whose circulation is intact, he comments on the fact that in many of his tracings the height of the contraction, or rather tension of the muscle, diminished toward the end of a period of twenty-five minutes, while at other times there is a slight rise from the general fatigue level. He also says that the initial fall of the curve frequently goes beyond the ultimate fatigue level, to which it gradually returns. This is shown in one of his tracings (l. c., p. 390) where the experiment lasted one hour. I have at times seen both these forms of curve, though apparently in a smaller percentage of cases than Schenck. Many secondary factors, such as afferent impulses from the tendons and joints, the interference with maximal volitional innervation from the tediousness of the experiment or the attraction of the attention from the work, the slight change of leverage due to the slipping of the splint on the finger, and even the conscious sensations of fatigue from the working muscle itself, are known to affect the height of contraction in these experiments, apart from the conditions of fatigue in the muscle itself. Moreover, curves are very frequently obtained which show no such variations from the type I have described and to which all tracings more or less conform. We need not, therefore, hesitate to assert that the aberrant character of the curves in question is due to quite secondary factors and not to simple fatigue. We may express the matter somewhat differently, as follows: whenever the influence upon the nervous system of afferent impulses from peripheral non-muscular organs, such as tendons and joints, is absent or completely neglected, and the mechanical factors of fixation of the hand and attachment of the resistance to the finger remain constant, maximal volitional innervation with each contraction will give an asymptotic curve of fatigue; deviations from this type are not phenomena of pure neuro-muscular fatigue, but are due to secondary and often unavoidable factors.

The tracing reproduced in Fig. 7 shows the effect of variations in

[1] Schenck: Archiv für die gesammte Physiologie, 1900, lxxxii, p. 384.

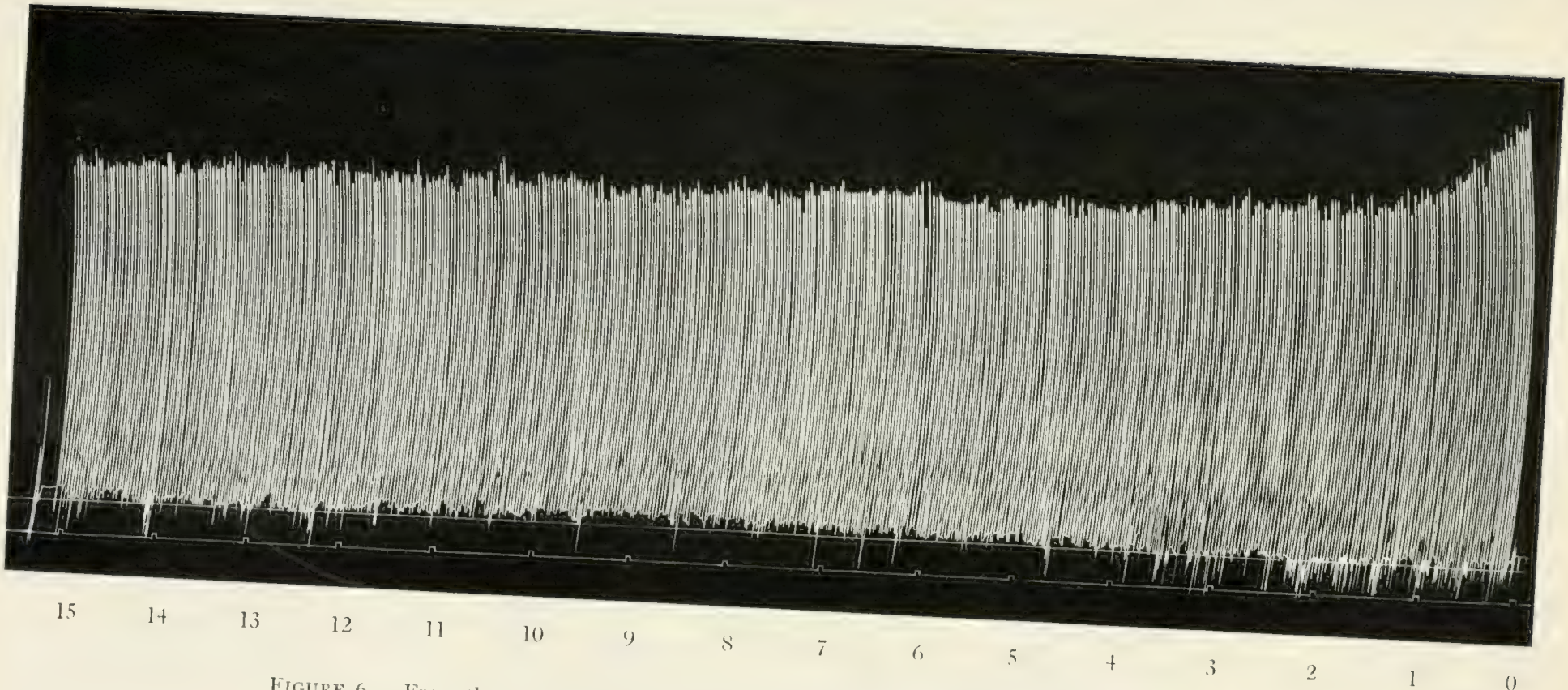


FIGURE 6. — From the experiment of May 15, 1899. Time record in minutes. Rhythm $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.}}{\frac{3}{2} \text{ sec.}}$

rhythm upon the resulting curve. It will be observed that on passing from the more rapid to the slower rhythm, the height of contraction increases in an asymptotic curve until a new and higher fatigue level is established. If we now return to the former rhythm, the curve falls in the same general manner as at first, ultimately establishing the same fatigue level as before. The level of the rhythm $\frac{C}{R} = \frac{1 \text{ sec.}}{9 \text{ sec.}}$ or $\frac{\frac{1}{2} \text{ sec.}}{\frac{1}{2}^{9} \text{ sec.}}$ is the same as the height of the original contraction. This agrees with the experience of earlier observers working with the weight ergograph. It is significant, however, that in an experiment like the one from which the tracing in Fig. 7 was taken, although the height of contraction with the slower rhythm is as high as the initial contraction of the experiment, the curve frequently falls more swiftly to the fatigue level on again passing to the more rapid rhythm than it did at first, although the fatigue level actually established is the same in the two cases. (See page 255.)

Fig. 8 gives a similar tracing of recovery from volitional tetanus, showing the same character of recovery upon passing to a rhythm giving longer periods of rest between contractions.

Most workers with the ergograph have noticed the tendency of the curve to assume a more or less rhythmic character; indeed it rarely happens that we obtain a perfectly even series of contractions, although the tracings given in this paper show that in a trained muscle the variations may be very slight in amount. Lombard[1] especially has studied these variations in successive contractions, and has found that they may be very large at times. In my own case they were much smaller; at first sight at least, for the weight instrument necessarily exaggerates them whenever the weight approaches the maximum for the fatigued muscle (see page 252). In all cases it seems to the writer that these variations are to be explained by other causes than simple fatigue; in other words, they are not characteristic of the fatigue of the simple neuro-muscular mechanism. Undoubtedly a large number of such cases can be traced to one of two causes. They were much more marked, in the first place, when the movement consisted of flexion of all three phalanges, than when it consisted of flexion at the first phalangeal joint. This, of itself, would suggest that the factor of coördination has much to do with the result; as such the variations may be properly regarded as

[1] LOMBARD: Journal of physiology, 1893, xiv, p. 97.

a fatigue phenomenon, but not one of simple fatigue. I have noticed the same increase of irregularity in the curve, in the second place, where the volitional innervation was evidently not maximal with every contraction. At times one becomes distinctly conscious of waves of volitional effort, because of the attraction of the attention to other things, from the difficulty of maintaining attention when the work becomes unusually monotonous, or from other causes. When, for one or another of these reasons, the will fails to give the maximal stimulus to the simple neuro-muscular mechanism, the latter fails to make its maximum effort and recovers to that extent from the state of fatigue indicated by the previous height of contraction; in such cases should the next volitional impulse be maximal, the resulting contraction will not only reach the height of the former fatigue level, but will go beyond it, in virtue of the recuperation caused by the submaximal character of the previous contraction. It is easy to see, moreover, that where two or three incomplete efforts have followed one another, this recovery may be considerable and so impress a very marked rhythm on the curve of fatigue. Looking at the matter from the standpoint of the simple neuro-muscular mechanism, the rhythmic character of the curve cannot be regarded as an expression of the fatigue of this mechanism, but of its unequal stimulation. Nor is it always the expression of central fatigue, properly so called, as when, for example, the inequality of volitional effort is due to the failure to give undivided attention to the experiment. Lastly, this rhythmic character of the curve is most marked in those who are beginning to use the instrument, and for obvious reasons. Certainly ergographic experiments are of little or no value in studying the details of simple fatigue unless the subject is thoroughly trained to the use of the instrument.

Perhaps nothing stands out so clearly in my results as the almost invariable establishment of an approximately constant average level of contraction. This appears in fully ninety per cent of my tracings from muscles in training. We are evidently dealing with the same thing observed by Treves, who found that by substituting smaller and smaller weights as fatigue progressed, a weight can finally be found which produces no fatigue whatever. With the spring it would seem that the muscle finds this weight itself. Moreover, many of Lombard's curves, especially some of those from the abductor indicis, show essentially the same thing. Figures 5 and 6 of his Plate I,[1] apart

[1] LOMBARD: Journal of physiology, 1893, xiv.

from the minor variations in height of contraction, are of the asymptotic character, as are also his tracings from a muscle with electrical stimulation.[1] Nor do the variations in the height of individual contractions seen in Lombard's tracings indicate the marked differences in working capacity which might at first sight be supposed, for the record of the weight instrument exaggerates the fatigue as the point is approached at which the muscle is just able to lift the given weight. For example, a fatigued muscle may be able to lift a weight of four kilogrammes only one centimetre; the work done would then be 0.04 kilogramme-metres; if the weight had been instead three kilo-

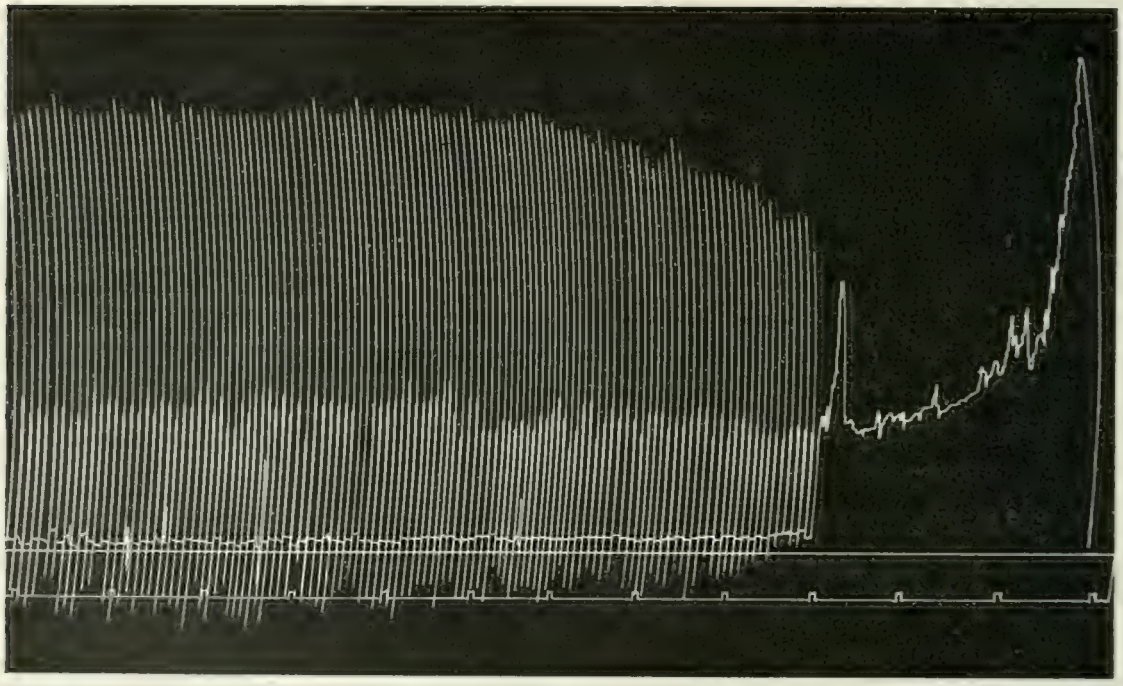


FIGURE 8. — Tracing from experiment of January 17, 1900. Time record in minutes. Rhythm of the contractions succeeding the volitional tetanus $\frac{C}{R} = \frac{\frac{1}{12}\text{ sec.}}{\frac{1}{24}\text{ sec.}}$

grammes, the muscle would probably have lifted it to the full height, say ten centimetres, doing 0.30 kilogramme-metres of work, although in exactly the same state of fatigue. Evidently under these conditions the work which a muscle will do is not a reliable measure of fatigue. Nor is this all; a very slight recovery of power would enable the muscle to lift the heavier weight to a much greater height, say five centimetres, thus doing 0.20 kilogramme-metres of work; and yet the condition of the muscle as regards fatigue in the two cases would not be represented by the quantities 0.04 and 0.20. Precisely the same

[1] LOMBARD: Journal of physiology, 1892, xiii, plate II.

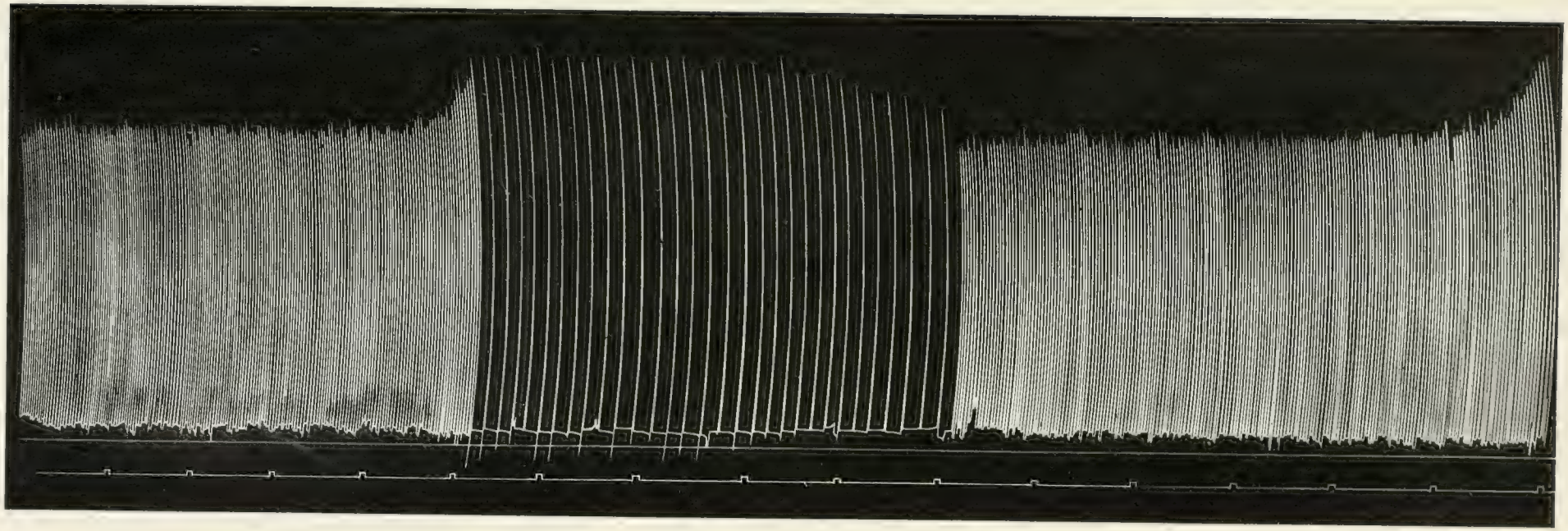


FIGURE 7. — Tracing from experiment of May 5, 1899. Time record in minutes. From 4.46 to 4.52 P. M. the rhythm was $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.}}{\frac{3}{2} \text{ sec.}}$; it was then changed to $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.}}{\frac{1}{2}\frac{9}{3} \text{ sec.}}$; at 4.57 it was again changed to $\frac{C}{R} = \frac{\frac{1}{2} \text{ sec.}}{\frac{3}{2} \text{ sec.}}$

thing is seen in the behavior of an isolated muscle which has been rhythmically stimulated to the point of exhaustion for the weight which it carries. The curve of fatigue is at first quite regular, falling, as Kronecker has shown, in a perfectly straight line. But I have never seen this regular fall continue to the base line despite every precaution to insure constant strength of stimulation; as the point is reached where the muscle is no longer able to lift the given weight, the curve always becomes irregular, the weight every now and then being lifted to a considerable height by individual contractions.

Turning for the moment to considerations of the theory of fatigue, I think it is evident that the establishment of a level is what we should expect. The one indisputable cause of ordinary fatigue thus far established is the presence of waste products of activity; it would seem reasonable to suppose that these limit the expenditure of energy (and so the work done) in proportion to their amount. At the same time, under the conditions of our experiments, the blood is removing them; and it would seem that when the rhythm is $\frac{C}{R} = \frac{1 \text{ sec.}}{9 \text{ sec.}}$ the removal is complete, no fatigue appearing in the tracing. As the rhythm becomes more rapid, these wastes are not completely removed between contractions; they gradually accumulate, and limit more and more the expenditure of energy and so the work done, until ultimately an equilibrium is established in which just as much waste is produced with each contraction as is removed before the next contraction. Under these circumstances a fatigue level of work is established, the height of which varies inversely with the rapidity of the rhythm. If the diminution in explosive fuel substance is also a cause of ordinary fatigue, plainly this factor would act in the same manner, the level being established as soon as the muscle manufactures from its reserve material or from the blood just as much fuel as it consumes with each contraction.

In previous ergographic work too much attention has been paid to the first portion of the curve and too little to the ultimate level established. The total number of contractions has seldom exceeded one hundred, and generally falls far below this. For this reason the establishment of a constant level of fatigue has almost if not entirely escaped attention. Nor is this all; in experiments with the constant weight, the weight has usually been chosen purposely so heavy as to bring the curve to the base line within forty or fifty contractions. When this is done it is obvious that the experimenter is working only

with the initial fall of the curve as seen in the experiments of Schenck and myself, and is neglecting one of the most important, if not the most important of its features, the fatigue level.

The time required to reach the fatigue level varies considerably in different tracings. In general about one hundred contractions $\left(\frac{C}{R} = \frac{\frac{1}{2}\text{ sec.}}{\frac{3}{2}\text{ sec.}}\right)$ were required in my own case. Sometimes as many as four hundred were necessary, while I find some eighteen or twenty tracings in which it was reached in less than one hundred.

It is one of the disappointments of my work that I have been unable to account satisfactorily for these variations in the rate of fall to the fatigue level, and especially so as it is a fundamental question in all ergographic work whether the fatigue level or the time required to reach this level is to be taken as the measure of the working condition of the muscle. It seems to the writer that neither one of these things alone can serve as the measure of fatigue or working capacity. One of the most rapid establishments of fatigue level I have ever seen is that of the experiment of Feb. 13, 1900 (see Table II, p. 260,) where only one minute was consumed (30 contractions), but where the level established was one of the highest; on the other hand the decline in the height of contractions is frequently of very long duration in the early experiments of the series, while the fatigue level may be very low indeed; at still other times the curve may fall very slowly to the same fatigue level, high or low, which it reaches rapidly on the following day, although the conditions of the experiment were, so far as could be seen, the same in the two cases.

The only factor which seems to be in any way connected with this variation in the curve of fatigue is the time of day at which the experiment was made, so that this matter may be profitably discussed in this connection. It is very difficult to eliminate the factor of training in tabulating the results with regard to this point, especially as no one series contains a sufficient number of observations to make averages reliable. The following table, however, which includes all the first experiments made each day during the month of May, 1899, is suggestive. Some seventy tracings, each of at least twenty minutes' duration had been taken during the preceding four months, so that no week had passed without at least two experiments; generally about four were made weekly and sometimes more. On the first of May the muscle may therefore be regarded as trained.

The table shows that the fatigue level seems to be quite independent

of diurnal variations, as does also the height of the initial contraction. It will be observed, however, that the fall to the fatigue level took place more slowly in the experiments made in the forenoon than in those made in the afternoon. This is suggestive as far as it goes,

TABLE I.

Date. 1899.	Height of first contraction. mm.	Fatigue level. mm.	Duration of fall to fatigue level. secs.
May 7. 9–10 A. M.	99	73	210
" 8. "	100	72	180
" 15. "	100	74	240
" 22. "	103	77	270
May 11. 10–11 A. M.	98	75	240
" 21. "	102	79	240
May 4. 11–12 M.	101	75	60
" 9. "	102	73	120
May 10. 12–1 P. M.	100	70	180
Luncheon usually between 1 and 2 P. M.			
May 16. 2–3 P. M.	98	76	100
May 3. 3–4 P. M.	100	78	150
" 23. "	98	76	120
May 5. 4–5 P. M.	100	78	60
" 19. "	95	74	200 (or less?)

but the observations are not sufficiently numerous to justify final conclusions.

My experience, with the trained muscle of course, thus fails to bear out Franz's conclusion that, " under similar objective conditions the daily variation is large." On the contrary I have been surprised

at the comparatively small amount of these daily variations. The fatigue level in the thoroughly trained muscle varied between 85 per cent and 60 per cent of the initial contraction, and different degrees of training evidently account for a large part of this variation since three-fourths of the experiments, and those occurring together in point of time, show variations only between 70 per cent and 80 per cent. (Compare protocol, Table II., page 258). The lower level almost always occurred in the earlier experiments of a series, the level gradually rising from day to day, and the higher level is confined to the series of January–February, 1900, when the muscle showed the highest degree of training. I am inclined to think that the greater uniformity of my results is due partly to the training afforded by the greater duration of individual experiments, and partly to the more successful elimination of the factor of coördination, since Franz uses not only the flexor but also the lumbrical muscle. Dynamometer strength tests show marked variations in the same way, these likewise being a measure not only of muscular strength but also of the power of coördination.

It will also be seen that my results do not agree with Franz's conclusion that "the amount of work that can be accomplished . . . is about 40 per cent as great at the end of a series of 150 contractions as at the beginning."[1] As I understand this statement, the writer seems to regard it as an approximation to a general law of fatigue. Reference to Table II. will show at once that such is by no means the case.

The fatigue curve of volitional tetanus. — Franz and Schenck have each described the results of experiments with volitional tetanus maintained against the resistance of a spring. Their results agree in general with those I have obtained and show that the curve of fatigue in tetanus corresponds essentially with that of fatigue with rhythmic contractions, representing indeed the limit toward which approach the curves with increasing rapidity of rhythm. One of my tracings is given in Fig. 8, to facilitate comparison with my other tracings.

The holding of a maximal volitional tetanus generally becomes exceedingly painful after the first minute, and sometimes sooner, the pain being located in the working muscle. This of itself explains the irregularities shown by the curve, as well as the fact that it rarely establishes a perfectly uniform fatigue level.

Second wind. — The phenomena of "second wind" have always

[1] Franz: This journal, 1900, iv, p. 372.

TABLE II.

Three series of experiments with the trained muscle. $\frac{C}{R} = \frac{\frac{1}{2}\text{ sec.}}{\frac{3}{2}\text{ sec.}}$

Date.	Exp.	Time.	Height of first contraction. mm.	Height of fatigue level. mm.	Duration of fall to fatigue level. secs.		Remarks.
Series I. 1899. Hook on splint $1\frac{1}{4}$ inches from joint.							
March 16		12 M.	106	65	400		
" 31		4 P. M.	103	90	30		
April 1		11 A. M.	95	83	320		
" 4		11 A. M.	102	84	360		
" 8		12 M.	105	81	140		
" 18		10 A. M.	110	83	120		
" 19		11 A. M.	108	83	180		
" 28		4 P. M.	104	84	90		
Series II. 1899. Hook on splint $1\frac{3}{8}$ inches from joint.							
May 3	I	3.50	100	78	150		
	II	4.23	100	74	60	*a*	Each of these experiments consisted of three parts; in part *a* the rhythm was $\frac{C}{R} = \frac{\frac{1}{2}\text{ sec.}}{\frac{3}{2}\text{ sec.}}$; the rhythm was then changed for five minutes approximately to $\frac{C}{R} = \frac{\frac{1}{2}\text{ sec.}}{\frac{1}{2}^{9}\text{ sec.}}$; the rhythm was then changed to $\frac{C}{R} = \frac{\frac{1}{2}\text{ sec.}}{\frac{3}{2}\text{ sec.}}$ with the result given in *b*.
			95	65	70	*b*	
" 4	I	11.06	101	75	60	*a*	
			98	76	30	*b*	
" 4	II	12.17	97	75	120	*a*	
			96	71	60	*b*	
" 5	I	4.23	100	74	60	*a*	
			95	65	70	*b*	
" 5	II	5.20	93	75	180	*a*	
			87	72	30	*b*	
" 7	I	9.40	99	73	210		Smoke experiment.
	II	10.24	94	68	300		

TABLE II (*continued*).

Series II (*continued*).

Date.	Exp.	Time.	Height of first contraction. mm.	Height of fatigue level. mm.	Duration of fall to fatigue level. secs.	Remarks.
May 8	I	9.40	100	72	180	
	II	10.23	94	66	250	
" 9	I	11.06	102	73	120	All experiments of this and the third series were made between 9 A. M. and 6 P. M.
	II	12.08	100	67	230	
	III	12.42	88	64	180	
	IV	3.06	85	65	120	
	V	3.45	100	70	90	
" 10		12.36	100	70	180	
" 11	I	10.54	98	75	240	
	II					Tracing lost.
	III	12.21	93	71	180	
	IV	2.50	98	71	180	
	V	3.30	—	72	60	
" 15	I	9.17	100	74	240	
	II	9.50	100	75	100	
	III	10.20	100	76	200	
" 16	I	2.50	98	76	100	$\frac{C}{R} = \frac{1 \text{ sec.}}{1 \text{ sec.}}$
	II	3.50	102	70	180	" "
" 17	I	4.03	100	—	—	$C = \frac{1}{2}$ sec.
	II	5.00	100	—	—	$R = \frac{9}{2}$ sec.
" 18		11.38	105	68	350	Muscle became slightly sore afterwards.
" 19		4.40	95	74	200?	Difficult to determine exact beginning of level.

TABLE II (*continued*).

Series II (*continued*).

Date.	Exp.	Time.	Height of first contraction. mm.	Height of fatigue level. mm.	Duration of fall to fatigue level. secs.	Remarks.
May 21	I	10.48	102	79	240	Smoke experiment.
	II	11.47	103	80	480	
	III	12.25	100	80	420	
	IV	1.05	98	78	150	
" 22	I	9.30	103	77	270	
	II	10.10	101	83	120	
" 23	I	3.12	98	76	120	
	II	3.52	99	75	90	
" 24	I	11.25	100	—	—	$\frac{C}{R} = \frac{1 \text{ sec.}}{9 \text{ sec.}}$
	II	12.03	100	—	—	

Series III. 1900. Hook on splint $1\frac{3}{8}$ inches from joint.

Jan. 18		4.15	98	81	120	
" 30		12.40	100	82	260	
" 31		12.45	100	80	240	
Feb. 4	I	12.05	102	81	240	
	II	12.40	100	75	300	
" 13		3.50	105	92	60	
" 21		3.07	102	—	—	
" 22		12.45	102	78	300	
March 2		4.40	105	—	—	

been an attractive field of speculation among physiologists. These phenomena, of course, occur in connection with highly coordinated activities, notably of the circulatory and the respiratory systems, and it is *a priori* probable that they are the indication of some temporary lack

of adjustment between the workings of the different physiological units involved. At the same time I am not aware of any experimental evidence which excludes the view that similar phenomena may at times be seen in the work of an isolated physiological unit, such as a muscle or nerve cell. In commenting upon the slight recovery observed at times by Schenck in his fatigue curves, I pointed out that their explanation is probably to be sought in certain experimental errors which have not as yet been entirely eliminated. The two most important of these result from the failure to maintain maximal volitional stimulation throughout the tracing (see page 249) and some slight change of leverage, as when the splint slips nearer to the axis of motion. Schenck himself comments on the presence of more or less painful muscular sensations, which, reaching their maximum during the first half of the tracing, gradually wear away; and I am inclined to attribute the temporary lower level of the fatigue curve to the interference of these afferent impulses with maximal innervation rather than to the more pronounced fatigue conditions in the muscle itself; and especially so since such recoveries are of infrequent occurrence in my own experiments. In some cases where they occurred, actual measurement after the experiment showed that the splint had slipped as described. It is also a point of some practical importance that few metronomes will maintain a constant rhythm as the spring runs down. Whenever an experiment extends over ten minutes it is well to have the spring rewound; it has been my usual practice to do this every five minutes.

After all, the few recoveries observed are very slight in amount and fail to explain the very marked systemic phenomena of "second wind," which for the reasons given do not seem to be observed at all in the fatigue of the muscle fibre itself.

Closely connected with this is the fact, so well known, that any general bodily effort can be sustained with greater ease and efficiency, if we gradually "warm up" to the work; in such cases the phenomena of "second wind" are usually insignificant or wholly absent. I have compared the fatigue level established by gradually increasing the height of contraction over a period of four or five minutes until the maximum pull was reached with that observed on alternate days under the same conditions but with the exertion of maximal effort from the first. These tracings, five of each kind in number, have failed to show the slightest difference in the level established. We may, therefore, conclude that both "second wind" and "warming up

to work" are phenomena of adjustment of the various mechanisms of the organism as a whole; they are matters of coördination and not of simple neuro-muscular fatigue.

The Effect of Smoking on the Curve of Fatigue.

The considerations urged in the paragraphs immediately preceding must be applied to the comparison of my results with those of Lombard [1] with regard to the effect of smoking upon the curve of fatigue. This writer measured only the rate of fatigue, and that with flexion of the entire finger, which, we have seen, introduces a much greater amount of coördination into the conditions of the experiment. He found in his own case that a marked depressing influence resulted from smoking a cigar.

I have made three experiments substantially as follows. The tracing proceeded for the first ten minutes in the usual manner; in this time the fatigue level was established; a pipe or cigar was then lit without interrupting the work, and smoked during the remainder of the tracing (twenty minutes). No change whatever occurred in the height of the fatigue level. The splint was then removed to allow the effects of congestion to pass away, and twenty minutes later, the smoking being continued during this time as well as during the subsequent duration of the experiment, a new tracing was taken, lasting twenty minutes; after a second rest of twenty minutes this was repeated, this third tracing being followed in the same way by a fourth. The fatigue level remained practically the same in all four tracings, although the long continued smoking had exerted conscious physiological effects. Nor do the tracings obtained in these experiments differ from controls made on other days. One example will suffice (see Table III.).

It will be observed that the progress of fatigue was much more gradual during the smoking than it was on the following day, and it so happens that the same thing is true of the other experiments. This is interesting in connection with the popular impression that smoking enables certain persons, at least, to bear fatiguing work more easily; but three experiments are hardly enough to settle this point.

[1] Lombard: Journal of physiology, 1892, xiii, p. 44.

Do the Results of Alternate Electrical and Volitional Stimulation Differentiate the Fatigue of the Nerve Cell from that of the Muscle?

In Mosso's first paper on the results of ergographic work[1] he showed that when a muscle is exhausted by periodical electrical stimulation, the volitional stimulus is still effective, and *vice versa*. In the same paper he gives tracings which show marked recovery of the power of volitional contraction during electrical stimulation, but no recovery in the height of contractions from electrical stimula-

TABLE III.

Date. 1899.	Time. A. M.	Initial contraction. mm.	Fatigue level. mm.	Duration of fall to fatigue level. secs.
May 21	10.48	102	79	240
(Smoke)	11.47	103	80	480
	12.45	100	80	420
	1.05	98	78	150
May 22	9.30	103	77	270
(Control)	10.10	101	83	120

tion during a period of volitional stimulation. These two statements are somewhat difficult to reconcile, but without insisting upon this point at present, I desire to consider briefly the conclusion which Mosso draws, that no small part of the fatigue observed in the usual ergographic tracings is of central origin, the recovery in the height of volitional contractions during electrical stimulation being attributed to the period of rest which the nerve cells have enjoyed while the muscle is being stimulated by electricity. It is certainly remarkable that a muscle fibre which no longer responds to the electrical stimulus will give a good contraction with a nerve stimulus; and if we are to accept Mosso's interpretation of his results, we are forced at the same time to assume that there is some essential thing in the nature of the nervous stimulus which is not reproduced by the electrical

[1] Mosso: Archiv für Physiologie, 1890, p. 83.

stimulus, an assumption which is, of course, by no means impossible. These experiments have been widely accepted as proof that the nerve cell fatigues more rapidly than the muscle fibre. Mosso's observations were still further extended by Lombard,[1] who used in one very striking series of experiments alternate electrical and volitional stimuli (*loc. cit.*, Plate II.) which show quite independent curves of fatigue. The curve obtained from the electrical stimulation is of the same character as that obtained by Schenck and myself during volitional contractions, while Lombard's simultaneous curve of volitional fatigue shows many irregularities; here again the conclusion is drawn that the curve of central fatigue is quite different from and in general more marked than that of peripheral fatigue.

I have already pointed out that neither Mosso nor Lombard were dealing with the simplest neuro-muscular mechanism, since both writers flexed the entire finger with volitional contractions, and so used the coördinated action of the flexor and the lumbrical muscles; consequently the electrical stimulation did not act upon exactly the same complex of muscles as the volitional. There is, however, one obvious error which both writers seem to have disregarded, and yet which seems to me to give a sufficient explanation of their results. It is impossible to apply an electrical stimulus, either to a nerve or a muscle, by means of electrodes placed on the skin, in such a way as to insure equal stimulation of every fibre; the greater part of the current will pass directly from one electrode to the other, and the fibres which happen to be affected by this part of the current will be more strongly stimulated than those affected only by the loops of the current on either side. The very fact that the height of contraction with electrical stimulation was less than that of the volitional contractions suggests that this is exactly what occurred. Only part of the muscle was thus sufficiently stimulated, and the pull was equivalent to that of a weaker muscle; those fibres thus inefficiently stimulated, of course, recovered more or less from their fatigue, so that upon returning to the volitional stimulation (and so the stimulation of every fibre) more work was done. We thus see why there should be an apparent recovery of volitional power during direct or indirect electrical stimulation of the muscle.

With regard to the response of the muscle to direct or indirect electrical stimulation after exhaustion by volitional stimulation, I have already mentioned the apparent contradiction between this result and

[1] Lombard: Journal of physiology, 1892, xiii. p. 6.

others by the same worker (Mosso); I think, however, that the following considerations show that we should expect such a result in a certain number of cases. When a muscle contracts, the waste products of muscular (not nervous) activity affect, not only the muscle fibres, but also the ultimate intramuscular nerve fibres; undoubtedly a large part of muscular fatigue is due to this cause; the diminution in the height of contraction is due, not to the fatigue of central mechanisms, nor wholly to that of the muscle fibre itself, but to the inability of the poisoned intramuscular nerve fibres to deliver the full strength of stimulus to the muscle. Under these circumstances, direct stimulation of the muscle by electricity would again stimulate the fibres affected to full contraction; the height of the contraction is not so great as it otherwise would be because only a part of the fibres receive the maximal effect of the current. Similarly when the electrical stimulus is applied to the nerve, the muscle contracts because the nervous impulse is now many times stronger than that resulting from the maximal discharge of the nerve cell, and so reaches the muscle with sufficient strength to produce contraction. Greene[1] has shown by actual measurement of the current of action that direct electrical stimulation of a nerve can produce much stronger impulses than are required to produce a maximal contraction.

In view of these considerations it would seem that neither Mosso's nor Lombard's results justify the conclusion that the nerve cell fatigues more rapidly than the muscle fibre.[2]

Summary.

1. Both the weight and the spring ergographs have special advantages in studying various features of neuro-muscular fatigue.

2. Where flexion of the finger is the movement employed, it should be at the first interphalangeal joint. To flex the entire finger introduces an unnecessary and undesirable complication of nervous and muscular coördination into the experiment.

3. Where comparison is to be made of fatigue curves from the same individual, there must be accurate regulation of the leverage at which the muscle works.

4. Neuro-muscular fatigue must be sharply distinguished from changes in the height of contraction resulting from other causes,

[1] Greene: This journal, 1898, i, p. 116.

[2] Cf. Woodworth: This journal, 1901, v, p. iv.

such as afferent impulses from tendons, joints, and the congested hand.

5. The curve of fatigue in the trained muscle falls as an asymptotic curve to what is practically a constant fatigue level.

6. The height of the fatigue level varies inversely with the rapidity of the rhythm of contraction.

7. The rhythmic variations in most ergographic tracings are the result of errors of experiment. The more completely these errors are eliminated, the less noticeable are the variations.

8. In the trained muscle the variation in the height of the initial contraction and of the fatigue level may be and possibly always is very slight from day to day. Marked variations, on the other hand, occur in the time required to reach the fatigue level, and my results suggest that there is some relation between this and the time of day the experiment is made.

9. The phenomena of "second wind" and of "warming up to work" seem to be entirely absent from the work of the simple neuromuscular mechanism.

10. Smoking failed to produce any effect upon the curve of fatigue, except possibly to prolong the duration of the fall to the fatigue level.

11. The experiments of Mosso and Lombard on alternate electrical and volitional stimulation of muscles do not justify the conclusion that the nerve cell fatigues more rapidly than the muscle fibre.

THE ELECTRICAL RESISTANCE IN DYING MUSCLE.

By T. KODIS.

HERMANN, in an elaborate paper on the conductivity of living nerve and muscle,[1] found, among other facts, that dead muscle shows less electrical resistance than living muscle. Jolly, in 1884, came to the opposite conclusion. Both investigators worked with the constant current. But the constant current has several disadvantages, and, at present, physicists have almost totally abandoned this method in measuring the resistance of electrolytes. The constant current produces polarization at the electrodes, and thus adds to the normal resistance. In the animal tissue it also produces "internal polarization"; for after the primary constant current has passed for some time, the tissue shows a current in the opposite direction. In order to avoid this disadvantage, Alt and Schmidt,[2] in 1893, used a Franklin's current produced by the Holtz machine; and, in accordance with Hermann and in opposition to Jolly, observed that a living muscle showed more resistance than a dead one. Their investigation remained unfinished, so that we do not know whether this method gives correct results.

At present Kohlrausch's alternating current method is generally used for the measurement of resistance in electrolytes, and to this method we owe almost all that we know about the laws of conductivity in electrolytes. In order to diminish polarization at the electrodes Kohlrausch introduced the alternating current, which reduces the polarization to a minimum.

My own experiments were undertaken with a view to investigating more closely, with the help of Kohlrausch's method, the changes of the resistance in a muscle during the process of death. Electrochemical theories show that the change in the conductivity of the electrolyte is connected with changes in the number of its dissociated molecules. The increase of the resistance points to the decrease in the number of ions in the same volume, all other conditions remaining

[1] HERMANN: Archiv für die gesammte Physiologie, 1872, v, p. 223, and vi, p. 313.

[2] ALT and SCHMIDT: Archiv für die gesammte Physiologie, 1893, liii, p. 575.

the same. Hence by measuring the changes in the conductivity as death comes on, it is possible to obtain an insight into the physico-chemical changes connected with this process. For this purpose it is necessary to eliminate some conditions not always considered by former investigators. The resistance of the same muscle in different stages of death and not the resistance of *different* muscles should be measured, because there are individual differences in the muscles. It is also important to avoid such secondary changes in the tissue as tetanus, and its destruction by the alternating current, which would alter the electric conductivity. The contraction of the muscle should be kept at a minimum, or avoided totally; for it was found by Du Bois-Reymond [1] that muscles have a different conductivity in rest and during contraction.

The method employed was as follows: The gastrocnemius and the muscles of the thigh of large, and, if possible, uninjured frogs were put in a longitudinal direction into a cylindrical U tube, one inch in diameter, in such a way that they filled the whole tube, except two inches at either end, as closely as possible without artificial pressure. During the observation the tube was immersed in melting ice, and the measurement was taken only when the temperature of the muscle became constant. The ice was used in order to prevent change in the muscle after its preparation. For the same reason the living frogs were cooled in ice-water, and operated on at a low temperature. To avoid contraction during the measurement with the interrupted current the muscle was kept in ice-water. As the cooling was not always sufficient to prevent contraction of the tissue the weakest possible current was used. This lessened the danger of injury of the tissue by the current itself. The induction coil employed was very sensitive. It was provided with a small Wagner's hammer, which gave a very high and distinct tone in the telephone. The coil was connected with one or two Leclanché cells.

The current was applied to the muscle by electrodes constructed in the following manner. Two glass funnels, with tubes two inches long were fitted into the ends of a U tube (Fig. 1). The funnel tubes were closed by plaster of paris one fourth of an inch thick. The funnels were filled with a 0.7 per cent solution of sodium chloride. In all my observations the solution was kept at the same height in

[1] Du Bois-Reymond: Untersuchungen über thierische Electricität, 1849, ii, p. 74.

the funnels. In the upper part of the solution were immersed the platinated platinum electrodes, which are used in an ordinary Kohlrausch apparatus. Electrodes with large surfaces were necessary in order to avoid polarization. I was not able to obtain any constant results so long as I used small surface electrodes, applying them directly to the muscle.

After the first measurement the bridge was reversed and a second measurement taken. The known resistance was then changed, and the measurement taken again. The U tube with the muscles was then removed from the ice, and kept for a certain time in the temperature of the room, from 21° to 23° C. After the muscle had been some time dying, new measurements were recorded, the muscle being cooled as before. This cooling was necessary in order to have all observations at the same temperature, for a change in temperature affects the conductivity. The observations were repeated from time to time until the death of the muscle was complete. A lack of contraction of the resting muscle when stimulated with a strong current at normal temperature was considered to be proof of death.

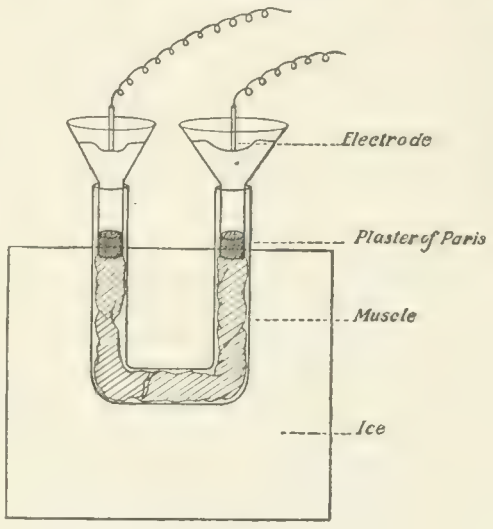


FIGURE 1.

In Table I are the results which I obtained. They include only such experiments as I considered exact and conducted under the most favorable conditions.

The analysis of the data obtained shows a very marked decrease of resistance as the death of the tissue progressed. This decrease of resistance is still more marked than is shown by the data, for the constant resistance offered by the electrodes, which amounted to about 10,000 ohms, must be taken into account. If we deduct this number from the figures of the table we find that the resistance decreases immediately after the death of the muscle to about one fourth the resistance of the living muscle. Further decrease of the resistance is due to the decomposition of a dead muscle. The coagulation of the muscle by heat decreases still more its electrical resistance, as is shown in Experiment IV.

TABLE I.

Experiment.	Temp. C.	Hours.	Resistance. Ohms.	Remarks.
I	+0.1°	0	36383	
	+0.1°	2¼	35416	
	+0.3°	5	30800	
II	+0.1°	0	42230	
	+0.2°	1	36977	
III	0	0	33936	
	+0.2°	3	30556	
	+0.6°	7	28115	
IV	+0.4°	0	57000	Another U tube.
	+0.6°	4	42612	
	+0.4°	23	34054	
	+0.4°	..	26511	Muscle coagulated by heat.
V	+0.4°	0	81000	
	+0.6°	2½	76000	
	+0.4°	5½	57000	
	+0.4°	24	23222	
VI	−6.0°	0	(200000)	Not exact; muscle is frozen.
	−3.5°	2½	187000	
	−0.5°	2	48553	Muscle melting.
	0	..	44200	Muscle was not heated in the intervals between the measurements.
	+1.5°	..	33720	
	+2.0°	..	29931	
	+2.5°	..	28141	
	+3.0°	..	27558	
	+7.4°	3	25941	

TABLE I (*continued*).

Experiment.	Temp. C.	Hours.	Resistance. Ohms.	Remarks.
VI (*cont.*)	+7.6°	..	25431	
	+8.0°	..	24417	
	+11.0°	..	23174	
	+11.5°	4	22989	
	+13.8°	..	21649	
	+14.0°	4½	21649	Muscle was heated.
	0.6	17	20425	
VII	−3.5°	0	56237	Another U tube. Muscle is frozen.
	+0.6°	4	44200	Muscle showing a slight contraction.

Clearly our experiments confirm the statement of Hermann, that there is a decrease of resistance in dying muscle. With the present notions of electrochemistry, however, it is impossible to agree with his explanation of this fact. Hermann [1] thought that the greater resistance of living muscle was due to the polarization which appears in the living tissue during the passage of the electric current. According to his view, the "polarity" diminishes in the dying tissue and consequently the resistance in this tissue appears smaller. The "polarity" in the living tissue was first discovered by Peltier. DuBois-Reymond [2] confirmed Peltier's observation and found that a current of about four volts passing through the tissue produces an opposite current of very marked strength. He also observed the same phenomenon after the passage of the electric current through porous bodies such as the cups used in Daniel cells. DuBois-Reymond's experiment has often been repeated by physicists and physiologists, especially by Hermann and Hering. DuBois-Reymond and Hermann explain the animal polarization by assuming that it is a process similar to the polarization at metallic electrodes, and consequently is due to the same cause, namely, the separation of the free ions from the

[1] Hermann: Archiv für die gesammte Physiologie, 1872, v, p. 232.

[2] DuBois-Reymond: Untersuchungen über thierische Electricität, 1849, i, p. 376, and ii, p. 377.

solution on the membranes of the tissue. Hering comes to a totally different conclusion. He finds that the animal polarization is nothing more than a result of the injury of the living tissue by the electric current and that the polarization current in animal tissue is nothing else than the current of rest.

The theory of DuBois-Reymond and Hermann does not agree with the modern electrochemical theories. It is now considered certain that ions do not separate from the solution except on the metallic electrodes. Therefore the opposite current, which was ascribed to the polarization, cannot be a result of the appearance of free ions on the membranes.

Since Hermann's theory was untenable, a new theory was advanced by Nernst [1] and Boruttau.[2] They suppose that the opposite current in the tissue is due to the change of the concentration of electrolytes in different parts of the tissue, resulting from the primary current. They also think that such a change of concentration takes place on the semipermeable membranes of the tissue. This theory is a very probable one; still the fact that we observe similar phenomena on the porous bodies shows that the semipermeability does not play any particular part in it.

In order to find more certain data in regard to the change of resistance caused by polarization, I measured the resistance of frozen muscles. It is known that freezing does not kill the muscle of cold-blooded animals, but preserves the morphological and chemical structure of the living tissue and at the same time suspends all properties of the living matter.[3] On the other hand the formation of ice in the electrolytes does not influence the conductivity otherwise than by the decrease of temperature; the resistance increases about 2 per cent for every degree Celsius of fall in temperature, without regard to the frozen fluid or undercooled state of the substance. The results which I obtained are illustrated by Experiments VI and VII of the table. The resistance of the living muscle does not decrease after it has been frozen, as would be the case were the larger resistance during life due to polarization. I consider that these experiments have proved that in the dying and dead muscle the number of ions is increased about one fourth.

[1] Nernst: Nachrichten von der Gesellschaft der Wissenschaften zu Göttingen: Mathematisch-physikalische Klasse, 1899, p. 104.

[2] Boruttau: Archiv für die gesammte Physiologie, 1899, lxxvi, p. 626.

[3] Kodis, T.: Centralblatt für Physiologie, 1898, xii, p. 593.

It is, of course, impossible at present to state what these ions are which appear in the tissue after death. I will venture to make a suggestion with regard to the inorganic salts. Chemical analysis shows a great quantity of K salts, especially phosphates, in muscle. The K salts cannot be free in the living muscle because they produce great muscular changes when applied for experiment. Biedermann [1] found that K_3PO_4 (and other K salts) in 1–2 per cent solution produce a current of rest in the muscle without further injury, and that the contractility of the muscle changes in such a way that it does not respond to the closing of the current when K salts are applied at the cathode. These phenomena disappear, the muscle is washed, and the salts removed. Exactly the same phenomena were evidenced by the following experiment. A cathode composed of dead muscle was applied to a living muscle. After a short time the muscle did not respond to the closing but only to the breaking of the current. In order to get a closing contraction I was compelled to take a much stronger current than before. A cathode of living muscle applied to living muscle has no influence upon contraction if the current remain of the same strength and density. The above experiment shows very conclusively, I think, that the K salts are combined chemically with colloids in the living protoplasm and that they become free as electrolytes during the death of the protoplasm.

These experiments were made in the physical and physiological laboratories of the University of Michigan in 1899, and I take this opportunity to express my thanks to Professor Lombard and Dr. Guthe for their valuable suggestions and for their kindness in offering me the use of their laboratories.

[1] Biedermann: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften zu Wien, 1880, lxxx, p. 367.

ON THE VARIATIONS IN THE SULPHOCYANIDE CONTENT OF HUMAN SALIVA.

By E. C. SCHNEIDER.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

ANY one who has had occasion to make frequent tests for the presence of potassium sulphocyanide in saliva must have become impressed with the extreme variations in intensity of reaction which the secretion from different individuals shows. These variations have been noted for many years, and the investigation of their cause, together with other problems concerning so unusual a secretory product as potassium sulphocyanide, has led to considerable difference of opinion. It is scarcely necessary to mention the older literature on the subject in detail, since this is referred to in the larger reference books on physiological chemistry, and has recently been reviewed by Fried. Krüger.[1] Various writers[2] have stated that the sulphocyanide is frequently absent from human saliva; others, again, have assumed on the basis of Schiff's observations that this compound is formed in the saliva on standing and is perhaps dependent for its origin on a decomposition of the fluid. Claude Bernard first called attention to the differences in the intensity of the reaction for sulphocyanide between the saliva of smokers and non-smokers; and in a careful investigation of this subject Krüger has observed that the mixed saliva of smokers uniformly contains two to three times more sulphocyanide than that of non-smokers. He could not agree with Schiff's conclusion regarding the post-secretory formation of the sulphocyanide — a result with which Grober's[3] recent observations coincide. And in describing an apparatus designed for the colorimetric estimation of sulphocyanide in saliva, Albert writes: "I have worked with it enough to be unable to confirm Schiff's state-

[1] KRÜGER: Zeitschrift für Biologie, 1899, xxxvii, p. 6; also GROBER: Deutsches Archiv für klinische Medicin, 1901, lxix, p. 243. The earlier literature will also be found discussed in LONGET: Traité de physiologie, 1868, i, p. 183 *et seq.*

[2] *E. g.*, HOPPE-SEYLER: Physiologische Chemie, 1881, p. 186; MOORE: Schaefer's Text-book of physiology, 1898, i, p. 345.

[3] GROBER: Deutsches Archiv für klinische Medicin, 1901, lxix, p. 246.

ment that the sulphocyanide increases in saliva that is kept a few hours owing to decomposition of the fluid. I can also say its existence does not in any way depend either on the presence of carious teeth or the use or non-use of tobacco." [1]

In view of the attention which has lately been directed towards the possible relationship between the excretion of potassium sulphocyanide and functional disorders of the body,[2] it seemed of interest to present some data which have been accumulated during the past two years regarding this constituent of the saliva from a large number of normal individuals.[3] In testing for the sulphocyanide, Solera's reaction as modified by F. Krüger[4] was found to be most delicate, and preference has been given to it over the older ferric chloride test. Filter paper of good quality is saturated with a half per cent starch paste containing a little pure iodic acid in solution, and is then dried in the air. When carefully prepared, this test-paper responds readily to very small quantities of sulphocyanide by giving a blue coloration due to the liberation of iodine; and with proper precautions the reagent-papers can be preserved without decomposition for a considerable time. In order to obtain some idea of the quantitative relationships of the reaction with specimens of saliva, solutions containing known amounts of potassium sulphocyanide were used in comparison. This method — also due to Krüger — was found to be quite satisfactory and reliable, as will be seen by the comparisons which were instituted with accurate determinations made by the method of I. Munk.[5] Thus the average potassium sulphocyanide content of the mixed saliva of a number of individuals was 0.003–0.004 per cent as indicated by the test-paper method, and 0.0032 per cent by Munk's method. Again, in the saliva of one individual the test-paper indicated 0.003 per cent potassium sulphocyanide; by Munk's method there was found 0.0025 per cent.

Almost all of our observations have been made on the saliva of

[1] Albert: The Lancet, February, 19, 1898, p. 496.

[2] *Cf.* Fenwick: The saliva as a test for functional disorders of the liver, London, 1889 (quoted by Gamgee: Physiological chemistry, 1893, ii, p. 20); Muck: Münchener medicinische Wochenschrift, 1900, No. 50, p. 1732; Grober: *Loc. cit.*, p. 243.

[3] Some of the results were presented by Professor Mendel at the meeting of the American Physiological Society, May, 1900.

[4] Krüger: Zeitschrift für Biologie, 1899, xxxvii, p. 15.

[5] I. Munk: Archiv für pathologische Anatomie, 1877, lxix, p. 350. This method is given by Gamgee: Physiological chemistry, 1893, ii, p. 55.

healthy young men (students in the laboratory); a few of the results were obtained from women and children. Among over two hundred and twenty-five individuals whose saliva was frequently tested, in but one case — that of a young man — could no reaction whatever be obtained directly by the Solera-Krüger method. After an interval of a year the sulphocyanide reaction was still absent in this individual, and its presence in his saliva could only be demonstrated by concentrating large quantities of the fluid. Krüger was able to detect at least traces of sulphocyanide in the saliva of almost all the young men examined by him. These results do not accord with the opinion of Hoppe-Seyler and others already quoted, regarding the frequent absence of sulphocyanide from human saliva.[1] Our observations are interesting in comparison with the data obtained by Grober[2] in an examination of one hundred patients in the Medical Clinic at Jena. These persons, men and women, had been non-smokers for several weeks at least. In eighteen cases no sulphocyanide could be detected in the saliva; in twenty-four cases traces were found, and eighteen yielded a small amount. Grober states that the sixty persons yielding these results were more ill in general than the remaining forty with whom stronger reactions for sulphocyanide were obtained. He concludes from these observations that the excretion of potassium sulphocyanide is presumably dependent upon the extent to which proteid utilization and katabolism proceed in the organism; since these processes are diminished in cachectic patients with severe chronic illness, the excretion of sulphocyanide, which is derived ultimately from proteids, must be slight or wanting.

Exact quantitative estimations of the sulphocyanide content have been made by Munk's method on the mixed saliva from twenty individuals. One hundred cubic centimetres of the filtered secretion were employed for analysis in every trial. The average result calculated from all these analyses is 0.007 per cent of potassium sulphocyanide which agrees closely with most of the results already published. However, when our determinations are classified into two groups according as they were obtained with the saliva of smokers or non-smokers, pronounced differences appear. The variations within the individual groups will be apparent in the following table:

[1] *Cf.* also Gamgee: Physiological chemistry, 1893, ii, p. 19.

[2] Grober: Deutsches Archiv für klinische Medicin, 1901, lxix, p. 243.

Determinations of KSCN in Mixed Saliva.

Smokers. Per cent.	Non-smokers. Per cent.
0.003	0.001
0.009	0.002
0.009	0.002 [1]
0.010	0.002
0.012	0.003
0.014	0.003
0.020	0.003
0.029	0.003 [1]
	0.003
	0.004
	0.004
	0.006 [1]
Average. 0.013	0.003

Observations made by the test-paper method already described have led to practically similar results. The reaction is designated as a "trace" when the color was weaker than that yielded by a 0.0016 per cent KSCN solution; a "weak reaction" is one stronger than that afforded on the same paper by a 0.0016 per cent KSCN solution, but less intense in color than the reaction with 0.008 per cent KSCN solution which marks the "strong reaction." Tests with the saliva of two hundred and twenty-nine individuals under a variety of conditions may be classified as follows:

KSCN Reaction in the Saliva.

	Smokers. Per cent.	Non-smokers. Per cent.
Traces (less than 0.0016 per cent) in	1	23
Weak Reaction (0.0016–0.008 per cent) in . .	23	72
Strong Reaction (more than 0.008 per cent) in	76	5

[1] Saliva from a woman.

No quantitative variations from these results were obtained with saliva from women and children. Our observations completely confirm those of Krüger who found 0.0117 per cent of KSCN in the combined saliva of a number of smokers, and only 0.0041 per cent in the secretion of an equal number of non-smokers. Analyses of saliva from single individuals were not made by him. Krüger also found a strong reaction in only seven per cent of all the non-smokers, as contrasted with seventy-two per cent of the smokers. Sex and age apparently played no part in this effect.

Grober has attempted to demonstrate the possible influence of tobacco on the sulphocyanide content of the saliva directly by allowing patients who had abstained from the use of tobacco for at least six weeks to smoke freely. Except in a very few instances he was unable to note any increase in the sulphocyanide. Minimal doses of HCN, however, seemed to be effective. We have made no systematic study of the direct effect of smoking. In the case of one young man, however, it was noticed that his reaction grew progressively stronger during a period of several weeks within which he gradually increased the use of tobacco; in a second individual who stopped smoking for ten days, the sulphocyanide reaction diminished noticeably during this period, but resumed its normal intensity when smoking was again continued. We have found the sulphocyanide content of the saliva of any individual in good health to be constant from day to day, and even over periods of several months.

Like several previous observers [1] we have found that the excretion of sulphocyanide may be considerably diminished by prolonged stimulation of the salivary glands. For example, in one case when the flow of saliva was continuously provoked by chewing a piece of soft paraffin for three hours it diminished as follows:—

At	8.15	the saliva contained	approximately	0.004	per cent	KSCN.
"	10.00	"	"	0.003	"	"
"	11.00	"	"	0.002	"	"
"	12.00	"	"	0.002	"	"

This observation was frequently confirmed in other cases. No constant relationship between the content of sulphocyanide and the composition of the saliva (organic matter, ash) could be ascertained in a series of estimations made with this point in view.

[1] *Cf.* Longet: Traité de physiologie, 1868, i, p. 191; Grober: *Loc. cit.*, p. 243 (in ptyalism).

Muck[1] has lately demonstrated the presence of sulphocyanide in the fluid which bathes the conjunctiva, as well as in the secretion from the nasal mucosa. He obtained the reaction in individuals with catarrhal as well as healthy membranes, and observed that its intensity varied with that of the saliva. We have frequently obtained a strong sulphocyanide reaction with the secretion from the nasal passages in acute catarrhal conditions, and determined to ascertain whether a contamination with this secretion ordinarily determines the nature of the reaction with the saliva. This could readily be learned by examining the secretion collected directly from the ducts of the salivary glands. There are few previous observations on this point and they are in part contradictory. Mitscherlich[2] reported that the saliva from a fistula of Stenson's duct in man contained 0.03 per cent of KSCN; and Oehl[3] likewise stated that the parotid saliva of man was richer in KSCN than either the submaxillary or mixed saliva. In the submaxillary saliva of man Oehl and Sertoli found small quantities of sulphocyanide, although Eckhard had failed to detect it.[4] Longet[5] states that he obtained a reaction with saliva from the sublingual gland also.

With the Solera-Krüger test-papers we have made observations on the relative intensity of the sulphocyanide reaction in the parotid and submaxillary saliva of over fifty individuals. The fluid was obtained by introducing sterilized glass cannulas into Stenson's and Wharton's ducts. While in the latter case the cannula was usually introduced far enough to reach beyond the opening of the duct of the sublingual gland into the Whartonian duct, it is possible that the submaxillary saliva was sometimes contaminated with traces of the secretion from the sublingual gland. Occasionally the flow of saliva was provoked by placing a drop of alcohol upon the tongue. *The parotid saliva has uniformly been found to be richer in sulphocyanide than the submaxillary saliva collected from the same individual at the same time.* The two corresponding glands, however, usually afford reactions of like intensity. The difference between smokers and non-smokers, noted for mixed saliva, was found to hold good also for the

[1] Muck: Münchener medicinische Wochenschrift, 1900, No. 34, p. 1168.

[2] Mitscherlich: Poggendorff's Annalen der Physik, 1833, xxvii, p. 338.

[3] Oehl: quoted from Maly: Hermann's Handbuch der Physiologie, 1881, v (2), p. 16.

[4] See Maly: Hermann's Handbuch der Physiologie, 1881, v (2), p. 18.

[5] Longet: Traité de physiologie, 1868, i, pp. 189–191.

secretion from the individual sets of glands. The observations may be summarized briefly as follows: —

Comparison of the Sulphocyanide Reaction of the Parotid and Submaxillary Saliva.

KSCN Reaction.	Smokers.		Non-smokers.	
	Parotid saliva.	Submaxillary saliva.	Parotid saliva.	Submaxillary saliva.
Trace.	None.	4	2	19
Weak.	4	13	22	5
Strong.	24	11	None.	None.

The fact that sulphocyanide has been detected, as mentioned, in other secretions than the saliva, and that no constant connection between the composition of the saliva and its sulphocyanide content has been ascertained, lends favor to the view that this substance owes its origin to processes involving the transformation of body proteid. In view of the constant differences which occur in the secretion of the various glands of the same person, however, the possible specific influence of the individual glands in the elaboration of sulphocyanide can no longer be overlooked.

In conclusion, the writer desires to acknowledge his indebtedness to Professor Lafayette B. Mendel, at whose suggestion and with whose kind assistance these experiments were made.

THE INHIBITION TIME OF A VOLUNTARY MUSCULAR CONTRACTION.

By ALLEN CLEGHORN and COLIN C. STEWART.

[*From the Laboratory of Physiology in the Harvard Medical School.*]

FICK[1] observed that the voluntarily contracted abductor indicis muscle could be made to relax reflexly upon the application of a direct electrical stimulus. With a sub-maximal voluntary contraction this effect was preceded by a primary increase in the contraction due to direct action on the muscle; but with a maximal contraction, relaxation alone appeared. Fick notes that the time between the stimulus and the relaxation is 0.09 second, and from this concludes that the relaxation is not due to any direct action, but to an inhibition produced in the nerve centres by the sensory impulse. He failed, however, to get relaxation unless the stimulus was applied directly to the contracted muscle.

Working in this same direction, Mosso[2] found with the ergograph that 0.2 second is the minimum time for the interference with the motor discharge by local electrical stimulation.

Waller[3] does not accept Fick's experiment as an example of inhibition, but, attacking the problem by various methods, finds support for the view that the electrical stimulus suppresses the voluntary contraction only by stimulating antagonistic muscles.

In our own experiments we have invariably obtained relaxation in a voluntarily contracted muscle (flexor sublimus) on the application of an induced current to the opposite arm, as well as with strong optical and auditory stimuli. Although Fick failed to get this result, it clearly supports his conclusion as to the nature of the relaxation, which appears to be a true inhibition of motor discharge. Waller would explain the phenomenon by a reflex contraction of antagonistic extensor muscles; but, as will be shown in the present paper, the reaction is much too slow to be produced by a simple motor reflex.

[1] FICK: Archiv für die gesammte Physiologie, 1887, xli, pp. 176–189.

[2] MOSSO: Archiv für Physiologie, 1890, pp. 89–168.

[3] WALLER: Brain, 1892, xv, pp. 35–64.

The apparatus employed in the present research was a slightly modified Mosso's ergograph. Fig. 1 illustrates the modification introduced, and explains the electrical connections and arrangement of the apparatus.

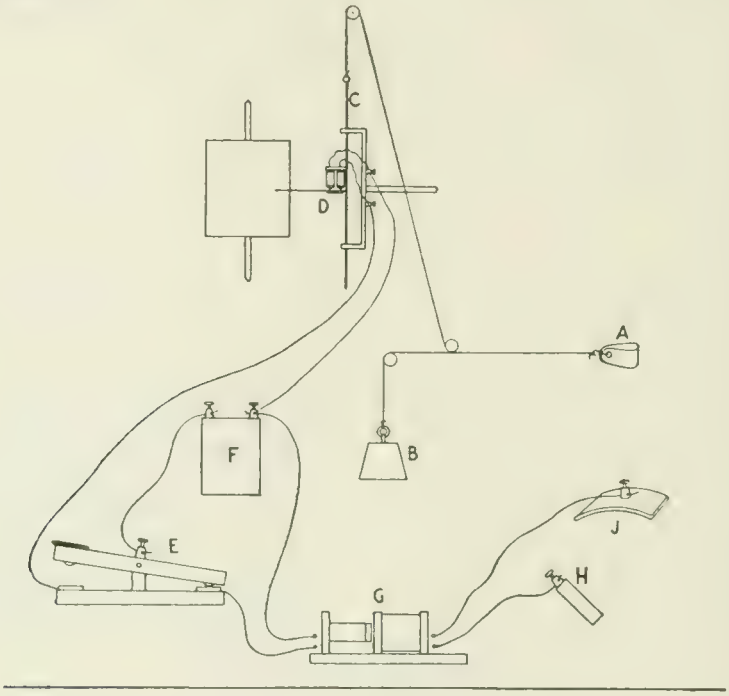


FIGURE 1. — A diagrammatic representation of the arrangement of the apparatus. The loop A into which the finger is inserted is connected with the weight B by a steel cord passing over a pulley. A second cord, passing over two pulleys, runs to the upper end of a vertical sliding lever, C, on the writing point of which is an electro-magnet, D. Reversing the two-way key, E, sends through the electro-magnet, D, the same current which before passed from the battery, F, to the induction coil, G. The secondary coil is connected with two electrodes; one, H, is grasped by the hand; the other, J, is fixed to the forearm. The distance of motion in the two-way key, E, is greatly exaggerated in the diagram.

The recording portion of the ergograph, as can be seen in Fig. 1 (C, D), differs from the usual form, a small electro-magnet being fastened to the movable rod of the ergograph. The armature of this magnet was in direct contact with the recording lever, which consisted of spring brass wire. This magnet was connected in such a way that on breaking the primary circuit by means of the key E, and thus inducing the stimulating current for the opposite arm, the current was thrown directly into the magnet. The armature at once elevated the pliable recording lever of the ergograph, already registering the contraction, and so marked in the course of the tracing the exact moment of stimulation. By the use of this system of registering the application of the stimulus, we avoided an extra tracing

(*i. e.*, that of an electric signal). Thus we were able to take about twenty records on one drum, as well as to facilitate greatly the measuring of the records. It became a simple matter to measure the distance between the rise in the tracing, drawn by the magnet, signifying the application of the stimulus, and the beginning of the fall produced by relaxation of the muscle. An attempt to measure the lost time in the working of the apparatus gave negative results, nor could we detect any inaccuracies in our observations which could be due to defective apparatus.

Our procedure was as follows: — The subject of the experiment, with his right arm fixed in the ergograph, at a signal lifted a known weight (2 kilos.) as high as possible by flexion of the middle finger.

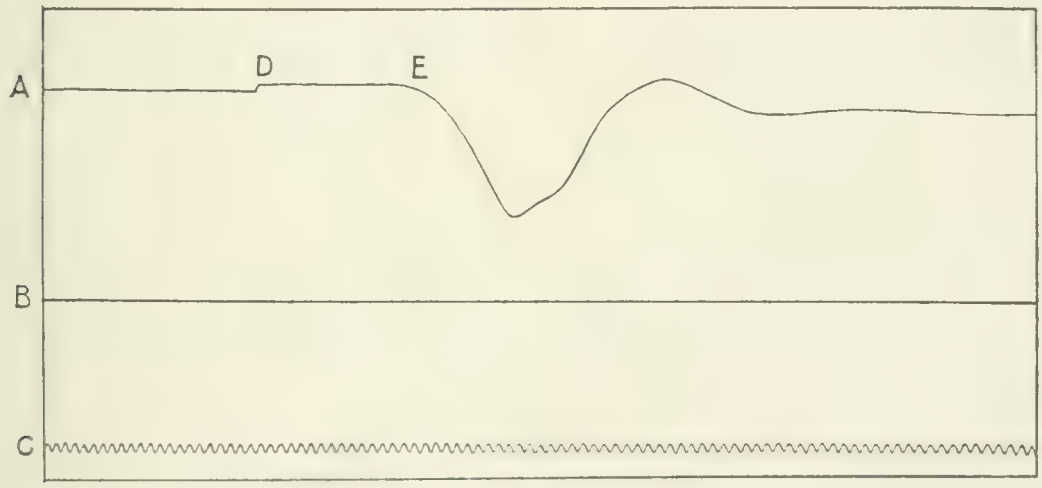


FIGURE 2. — Voluntary contraction inhibited by electrical stimulus applied to opposite arm. A, ergograph record, sustained contraction, weight 2 kilos; B, ergograph at rest; C, time marker, $\frac{1}{100}$ second; D, signal of stimulus; E, relaxation of contracted muscles.

This sustained contraction was recorded on a very fast drum, which also registered the movements of a tuning-fork vibrating one hundred times per second. While the subject maintained the contraction he received various strong sensory stimuli, — optical, auditory and electrical. The method of application of the two former was that described by Dr. Cleghorn.[1] The electrical stimuli consisted of strong single break induction currents applied to the skin of the opposite arm, as represented in Fig. 1. The application of the stimulus was registered on the drum in the manner described above, and was almost immediately followed by a sudden and great relaxation of

[1] CLEGHORN: This journal, 1898, i; pp. 336–345.

the contracted muscles. This is illustrated in Fig. 2, which shows a tracing selected from a considerable number as typical of the result obtained.

The subjects experimented upon knew neither the result expected, nor the time at which the stimulus would be made. We further controlled the results obtained by taking, with the same apparatus, the time of each individual's normal reaction to electrical stimuli. We have found by direct measurement of between 1000 and 1500 curves, taken from fifteen different subjects, that the time elapsing between the application of the stimulus and the relaxation of the muscle is considerably longer than the subject's reaction time. The relaxation is involuntary, and is not permanent; the subject quickly contracts the muscle again.

The following table shows the results of our experiments in actual time measurements ($\frac{1}{100}$ secs.). The three stimuli used have been classed by themselves. Under each is first given the number of observations made, then the average result of these observations, and the probable error.[1] The third column from the right gives the "general average," *i. e.*, the average of the results obtained from the three stimuli combined. Next we have the individual's "reaction time," which, it will be noticed, is in every case less than the "inhibition time," the last column showing this clearly.

Several minor points are worthy of note in connection with the above table: first, that in two subjects we obtained no result. We are unable to account for this, although it may also be mentioned that certain subjects gave a much more pronounced relaxation on the application of the stimulus than others. Second, the average inhibition time following the auditory stimulus is the longest of the three; next comes the optical stimulus, and lastly the induction current applied to the skin. The differences, however, are extremely slight and are not constant in the individual cases.

The demonstration of this inhibition, and the measurement of its time relations, in addition to bearing out the conclusion of Fick (*loc. cit.*) as to its true cause, are of interest in connection with the results of other authors, experimenting with various methods and for different purposes.

[1] The formula used by us to calculate the probable error in these observations was as follows

$$PE = \pm 0.845 \frac{\Sigma d}{n}$$

Individual.	Inhibition time (time in $\frac{1}{100}$ seconds).									General average.	Reaction time.	Inhibition minus reaction time.
	Stimulus.											
	Induction shock.			Sight.			Sound.					
	No. of observations.	Average.	Probable error.	No. of observations.	Average.	Probable error.	No. of observations.	Average.	Probable error.			
1	105	17.54	2.01	80	14.05	1.02	20	17.12	2.04	16.38	12.75	3.63
2	59	18.36	1.07	27	17.65	1.05	32	16.45	1.01	17.45	14.00	3.45
3	59	19.05	1.05	10	12.05	0.08	15	14.05	1.03	15.05	13.07	1.08
4	97	16.78	2.05	37	18.35	1.05	20	20.00	2.03	18.37	12.65	5.72
5	10	19.22	2.05	14	20.05	2.01	17	16.04	0.09	18.07	14.05	4.02
6	44	21.18	1.08	28	19.05	2.09	22	18.06	1.04	19.76	13.08	5.96
7	No result.	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
8	22	12.02	0.08	16	16.04	1.04	18	15.05	2.00	14.07	11.00	3.07
9	10	20.06	2.01	11	20.00	0.05	9	21.03	1.06	20.45	14.03	6.15
10	137	16.05	1.01	46	18.05	1.06	33	19.00	2.07	18.00	14.02	3.08
11	No result.	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
12	36	18.05	1.05	35	21.35	2.02	12	20.00	1.06	19.95	14.05	5.45
13	50	15.00	1.07	40	16.00	1.07	24	18.05	2.05	16.05	12.25	4.25
14	14	20.03	2.03	14	19.05	2.03	16	21.05	2.04	20.04	15.08	4.06
15	12	18.00	1.06	17	20.05	2.05	10	18.00	1.08	18.08	15.00	3.08
Total averages.	50.3	17.95	1.78	28.84	18.09	1.07	19.07	18.22	1.84	18.06	13.72	4.34

Bowditch and Warren[1] have shown that a voluntary contraction previously made in response to a signal alters the force of the knee-jerk, and that this effect varies with the time-interval. If the signal fell not more than four-tenths second before the knee-jerk, the result was augmentation. With an interval exceeding four-tenths second, the result was a decrease of force — a demonstration of the diffuse nature of the central changes following the reinforcing contraction. But from this four-tenths second, however, as the authors themselves point out, must be subtracted the reaction time elapsing between the signal and the reinforcing voluntary contraction which constitutes the true stimulus.

Broca and Richet[2] have demonstrated a similar diphasic reaction to stimulation in the cortex of the dog — the phase of inexcitability succeeding an augmentation phase lasting one-tenth second. Hofbauer[3] has obtained such variations in the force of a voluntary contraction. And an acceleration of the relaxation phase of a voluntary contraction has also been pointed out by Dr. Cleghorn.[4] This last result is distinct from the one which forms the subject of this paper. An acceleration of relaxation occurred because the stimulus, falling at the commencement of the process of relaxation, produced its primary augmenting effect. The present inhibition of a sustained contraction we believe to depend upon the fact that, as other authors have found, any sufficient activity of the nervous system is followed by a double wave of augmentation and depression of the condition of the nerve centres. The primary augmentation is of short duration, and does not increase the sustained voluntary contraction, because, on the application of the stimulus, the contraction is already maximal. The secondary depression, or inhibition, decreases the force of the motor discharge, either in the cord, or at a higher level, to an extent sufficient to produce a relaxation of the muscles involved in the contraction.

[1] Bowditch and Warren: Journal of physiology, 1890, xi, pp. 25–64.
[2] Broca and Richet: Archives de physiologie, 1897, pp. 864–879.
[3] Hofbauer: Archiv für die gesammte Physiologie, 1897, lxviii, pp. 546–595.
[4] Cleghorn: This journal, 1898, i, pp. 336–345.

THE COMPOSITION OF YELLOW FIBROUS CONNECTIVE TISSUE.[1]

By G. W. VANDEGRIFT and WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

Historical.

MOST of the animal tissues have been carefully analyzed and their general composition determined. We have not been able to find any record of such chemical study of ligament, however. Gorup-Besanez[2] mentions the fact that a few determinations of the composition of the middle coat of arteries, and several other forms of connective tissue containing elastic fibres, have been made, according to which the percentage of water varies between 57.5 per cent and 75.9 per cent. He doubtless refers to such incomplete analyses as those of the tunica intima and tunica media of the carotid artery, made by Schultze and quoted by Gautier,[3] as follows:

	Per cent.
Water	69.30
Elastin (including collagenous and cellular elements)	18.65
Other albuminoids	8.72
Extract in water-alcohol	2.27
Soluble salts	0.74
Insoluble salts	0.34

The functions of elastic tissues appear to be mainly of a mechanical nature, and there has been little to suggest that such forms of connective tissue as ligament contribute anything important in substance or effect to metabolism. Probably the seeming passivity, in the metabolic sense, of ligament and allied structures accounts for the lack of chemical attention they have received.

During Liebig's time, when elementary analysis was expected to throw much light on those transformations in the body which we now

[1] Reported, in part, before the American Association for the Advancement of Science, June, 1900: Proceedings, 1900, p. 123.

[2] Gorup-Besanez: Lehrbuch der physiologischen Chemie, 1878, p. 649.

[3] Gautier: Leçons de chimie biologique normale et pathologique, 1897, p. 297.

speak of as anabolic and catabolic, many of the tissues were given extended study.[1] Liebig, Scherer, Mulder, and many others, in those days, determined the elementary composition of muscle, blood, hair, cartilage, bone, tendon, and practically all of the other body parts (after desiccation), and gave empirical formulæ to these tissues just as they did to pure chemical substances. They deduced from these formulæ relationships and differences which were not particularly in harmony with observed functions, and which have not been borne out by subsequent research.

Scherer[2] determined the elementary composition of the dried middle coat of arteries. To this elastic tissue he ascribed the formula $C_{48}H_{76}N_{12}O_{16}$. Bergh[3] and Schwarz[4] have since made and analyzed several pure preparations of elastin from the aorta. The latter's studies of the composition and reactions of aorta elastin have led him to conclude that it is identical with the elastin of ligamentum nuchæ. The averages of the analytic percentage results obtained by these observers are here brought in contrast:

		C	H	N	S	O
Scherer.[5]	Tunica media	53.49	7.03	15.36	..	24.04
Schwarz.[5]	Purified aorta elastin . .	54.34	7.08	16.79	0.38	21.41
Bergh.	Purified aorta elastin . .	53.99	7.54	15.20	0.60	22.67

These results are sufficiently close in agreement to indicate chemically, as has been found histologically, that the tunica media of the main arteries is largely composed of elastin.

The earliest results of similar analysis which relate to ligament are, so far as we have been able to find, those obtained by Tilanus[7] and Müller[8] for ligamentum nuchæ, after extraction with water, alcohol, and ether by the first observer and with acetic acid, in addition, by the second. Tilanus gave his prepared tissue the formula $C_{52}H_{80}N_{14}O_{14}$. Numerous investigators have since analyzed elastin from the cervical

[1] Liebig: Die organische Chemie in ihrer Anwendung auf Physiologie und Pathologie, 1842, p. 320 *et seq.*

[2] Scherer: Annalen der Chemie und Pharmacie, 1841, xl, p. 1.

[3] Bergh: Zeitschrift für physiologische Chemie, 1898, xxv, p. 337.

[4] Schwarz: *Ibid.*, 1894, xviii, p. 487.

[5] Phosphorus and sulphur were not determined, but included (by difference) in the figures for oxygen.

[6] Compare with the analyses by Chittenden and Hart, p. 289.

[7] Tilanus: See Mulder, Versuch einer allgemeinen physiologischen Chemie, zweite Hälfte, 1844–51, p. 595.

[8] Müller: See Gorup-Besanez, *loc. cit.*, p. 140.

ligament, prepared by essentially the same process, but with more elaborate extractions. Comparison is made, in the following summary, of the latest analyses with Tilanus's and Müller's average results:

		C	H	N	S	O
Tilanus.[1]	Prepared ligament.	54.98	7.31	17.52	0.33	19.86
Müller.	Crude elastin . .	55.46	7.41	16.19	..	20.94
Chittenden and Hart.[2]	Pure elastin . . .	54.08	7.20	16.85	0.30	21.57

Analyses of Ligamentum Nuchæ.

In the analyses here to be described the results were obtained with ligamentum nuchæ, — a ligament composed in great part of yellow fibres and representing, perhaps better than any other part of the body, true elastic connective tissue.

Proportions of water, solids, organic and inorganic matter. — *Method of determination.* Perfectly fresh bloodless ligaments, taken from the animals immediately after their slaughter, were used. Within a few hours after removal from the body all adherent connective tissue was carefully cut off. The cleaned ligament was then divided into strips and very thin particles cut, from only the deeper portions of these, with scissors into weighed porcelain crucibles. This division of the tissue was made as minute as possible, and the process was carried out with the utmost rapidity to prevent loss of water by evaporation before the weight of tissue in use was determined. The weight of fresh tissue taken was determined by difference. The substance was then dried at 100–110° C. to constant weight, after which incineration was carefully conducted over a very low flame until all carbon was burned out and constant weight attained. No special difficulty was experienced in effecting complete combustion of the carbon over an ordinary Bunsen burner.

Analytic results. The tables on page 290 summarize the results of the general analyses of ligamentum nuchæ from the ox and calf.

Comparative results. — The data on page 290 show that the ligament of the full grown animal contains relatively less water and inorganic matter, and more solid substance and organic matter, than that of the calf, facts which are in entire agreement with comparative

[1] Phosphorus was not determined, but included in the figures for oxygen.

[2] Chittenden and Hart: Studies from the laboratory of physiological chemistry, Yale University, 1887–88, iii, p. 22. Compare with Schwarz's figures, p. 288.

Number.	Ligament used.	Percentage of fresh tissue.				Percentage of solids.	
	Grams.	Water.	Solids.	Organic matter.	Inorganic matter.	Organic matter.	Inorganic matter.
Ox ligament.							
1	5.47	59.34	40.66	40.26	0.40	99.02	0.98
2	4.34	60.34	39.66	39.28	0.38	99.06	0.94
3	7.89	58.58	41.42	40.86	0.56	98.65	1.35
4	8.96	58.46	41.54	41.11	0.43	98.96	1.04
5	7.64	56.36	43.64	43.18	0.46	98.94	1.06
6	4.49	57.37	42.63	42.13	0.50	98.83	1.17
7	4.22	56.32	43.68	43.17	0.51	98.85	1.15
8	3.22	55.39	44.61	44.17	0.44	99.01	0.99
9	3.29	58.10	41.90	41.45	0.45	98.93	1.07
10	3.94	56.42	43.58	43.05	0.53	98.79	1.21
11	3.92	56.55	43.45	42.96	0.49	98.89	1.11
Averages	5.22	57.57	42.43	41.96	0.47	98.90	1.10
Calf ligament.							
1	11.00	66.24	33.76	33.04	0.72	97.88	2.12
2	8.78	65.34	34.66	33.98	0.68	98.04	1.96
3	7.49	64.61	35.39	34.71	0.68	98.09	1.91
4	7.10	64.72	35.28	34.62	0.66	98.14	1.86
5	7.19	64.59	35.41	34.83	0.58	98.36	1.64
Averages	8.31	65.10	34.90	34.24	0.66	98.10	1.90

analytic results for other tissues of growing and mature animals. The summary on the opposite page contrasts the above average percentage figures with those for morphologically related parts:

	Ligament.		Vitreous humor.[1]	Costal cartilage.[2]	Bone with marrow.[3]	Adipose tissue; kidney fat.[4]
	Calf.	Ox.				
Fresh tissue.						
Water.	65.10	57.57	98.64	67.67	50.00	4.30
Solids.	34.90	42.43	1.36	32.33	50.00	95.70
Organic matter.	34.24	41.96	0.48	30.13	28.15	95.51
Inorganic matter.	0.66	0.47	0.88	2.20	21.85	0.19
Dry tissue.						
Organic matter.	98.10	98.90	35.29	93.20	56.30	99.80
Inorganic matter.	1.90	1.10	64.71	6.80	43.70	0.20

Inorganic matter. — The ash of ligamentum nuchæ contains chloride, phosphate, carbonate, and sulphate; also, sodium, potassium, calcium, magnesium and iron, the latter arising in all probability from minute quantities of blood held in the tissue capillaries.

Sulphate. — The sulphate reaction in our preliminary tests was decided enough to suggest unusual quantity. In numerous samples of ash obtained by burning in porcelain crucibles directly over gas flames we found 8.04 to 9.20 per cent of SO_3. Mörner[5] has lately called attention, in connection with the SO_3 content of bone ash, to the well known fact that, during incineration directly over an ordinary burner, sulphur is introduced in considerable proportion from the consumed gas. In ash made by incineration in platinum dishes over alcohol flames, however, we obtained the following results for SO_3, which were determined, in 0.2 to 0.6 gram portions after solution in hot dilute hydrochloric acid, by the usual barium chloride method:

[1] Representing jelly-like connective tissue. Analyses by LOHMEYER, source of material not specified. See GORUP-BESANEZ: *Loc. cit.*, p. 401.

[2] Human. Analyses by HOPPE-SEYLER. See KÜHNE: Lehrbuch der physiologischen Chemie, 1868, p. 387.

[3] Average of many analyses of various human bones before removal of marrow. HOPPE-SEYLER: Physiologische Chemie, 1881, p. 625.

[4] From the ox. ATWATER: Methods and results of investigations on the chemistry and economy of food, 1895, p. 34.

[5] C. TH. MÖRNER: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 311.

Percentage of SO_3 in ligament ash.

	1	2	3	4	Averages.
A	5.58	5.66	5.61		5.62
B	5.80	5.71	5.46	5.61	5.64
C	5.71	5.50	5.79	5.66	5.67
				General average . .	5.64

The above results are significant when compared with the following percentage figures for content of sulphuric acid in the ash of the tissues and fluids specified :[1]

Bone [2]	0.02	Liver	0.92	Serum	2.10	Bile	6.39
Muscle [3]	0.30	Lungs	1.40	Spleen	2.54	Cartilage [4]	37.47
Brain	0.75	Blood	1.67	Milk	2.64		

The unusually large proportion of SO_3 found in ligament ash undoubtedly arises from an organic source. The ash of blood and lymph, it will be seen, contains much less in proportion, as does also that of all the other tissues except cartilage. Attention has lately been called to the fact that mucin is contained in ligament in appreciable quantity.[5] We shall presently show that its percentage amount is about half that in tendon.[6] Mucin contains ethereal sulphuric acid, in a radicle very similar to, if not identical with, chondroitin sulphuric acid.[7] This latter body, and chondromucoid containing it, doubtless contribute the surprisingly large proportion of SO_3 to cartilage ash.[8]

[1] Most of these are taken from SCHÄFER'S Text-book of Physiology, 1898, i, p. 77.

[2] C. TH. MÖRNER: *Loc. cit.*

[3] WEBER: Quoted from HOPPE-SEYLER, Physiologische Chemie, 1881, p. 651.

[4] Calculated from HOPPE-SEYLER'S analyses as given by KÜHNE, Lehrbuch der physiologischen Chemie, 1868, p. 387.

[5] RICHARDS and GIES: Proceedings of the American Physiological Society, This journal, 1900, iii, p. v; also, *Ibid.*, 1901, v, p. xi.

[6] The greatest amount thus far obtained from normal ox tendon was 1 per cent. CHITTENDEN and GIES: The journal of experimental medicine, 1896, i, p. 186.

[7] LEVENE: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395.

[8] Bone ash contains only a trace, which has also been attributed to constituent chondroitin sulphuric acid. See C. Th. MÖRNER: *Loc. cit.*; also, BIELFELD: Zeitschrift fur physiologische Chemie, 1898, xxv, p. 350.

The unusual percentage of SO_3 in ligament ash must, it appears to us, be attributed, in much the greater part, to a similar source — that is, to the SO_3 radicle of the mucin, which, on burning, is transformed, in part at least, to sulphate.

Phosphate and chloride. — In view of the excessive amount of derived sulphate, determinations of the percentage quantity of other constituents in ligament ash could not be expected to give exact figures for proportionate content of inorganic matter in the fresh tissue. We have, however, determined phosphoric acid and chlorine, which appear to make up the bulk of the acid radicles. The former was determined by Mercier's modification of Neubauer's method,[1] in neutralized extracts of 0.5–0.8 gram of ash in 100 c.c., made by prolonged treatment with hot dilute hydrochloric acid. The latter was estimated by Mohr's method,[2] in aqueous extracts of 0.4–0.7 gram of ash in 100 c.c., made by continued heating on the water bath. The following percentage results were obtained:

		1	2	3	Average.
A.	P_2O_5	7.46	7.09	7.61	7.39
B.	Cl	29.16	28.91	28.79	28.95

These figures are all within the customary variations observed for other tissues. They suggest, of course, that chlorides are the predominant substances in the ash of ligament.[3]

Fat (ether-soluble matter). — Dormeyer's method[4] was used in these determinations. The percentage of water was ascertained for each sample dried to constant weight, and extraction of fat made from the pulverized dry material in quantities varying from 18 to 35 grams. The tissue used was taken from only the inner portions of the ligaments. The following percentage results were obtained:

	1	2	3	4	5	6	Average.
Fresh tissue.	1.26	0.94	1.03	1.45	0.89	1.17	1.12

The proteid constituents. — The chief organic substance in ligamentum nuchæ has long been known to be elastin. After Rollett's[5]

[1] Neubauer und Vogel: Analyse des Harns, zehnte Auflage, 1898, p. 731.

[2] *Ibid.*, p. 708.

[3] Bone contains only traces of chlorine (0.19% in the ash). Cartilage ash contains 3.70% of chlorine. See Halliburton in Schäfer's Text-book of Physiology, 1898, i, pp. 112 and 113.

[4] Dormeyer: Jahresbericht über die Fortschritte der Thier-Chemie, 1896, xxvi, p. 42.

[5] Rollett: Untersuchungen zur Naturlehre des Menschen und der Thiere (Moleschott), 1859, vi, p. 1. Also *Ibid.*, 1860, vii, p. 190.

researches on the structure of connective tissue, particularly tendon, it was assumed by various observers [1] that ligament contains representatives of the various proteids which Rollett identified. It was only recently, however, that particular attention was called to the fact that this representative of yellow fibrous tissue contains appreciable quantities of coagulable proteid, glucoproteid and extractives.[2] The quantities in which these substances are present make it probable that they are integral components of the tissue and not merely constituents of retained blood and lymph. Even after the finely divided tissue has been well washed in water, a process calculated to remove practically all lymph, these substances may still be separated from it in relatively large amount.

Coagulable proteid (*albumin, globulin*). The fresh cleaned tissue was cut into strips and these quickly torn into delicate shreds with forceps. 50–100 grams of the fibrous material were extracted, in each determination, with 200 c.c. of 1.25–5.0 per cent solution of sodium chloride, at room temperature for from three to four days. Powdered thymol prevented putrefactive changes. At the end of that time the extract was pressed through cloth, filtered, and the tissue thoroughly washed with water. The extract and washings were then heated to boiling. The coagulable proteids were completely precipitated on addition of a very small quantity of dilute acetic acid.[3] The precipitate was filtered on weighed papers, washed free from chloride with water, and the coagulated proteid determined gravimetrically after drying to constant weight at 100–110° C. The following percentage results were obtained in six determinations with samples from as many ox ligaments:

	1	2	3	4	5	6	Average.
Fresh tissue.	0.588	0.502	0.598	0.652	0.652	0.704	0.616

Mucin. — Rapidly shredded ligament, prepared as for the determinations of coagulable proteid, in portions of 100 grams, was extracted, with repeated shaking, in 250 to 300 c.c. half-saturated lime water for several days at room temperature. The glucoproteid was completely precipitated from the extract and washings on acidification with 0.2 per cent HCl. Its amount was determined, after filtering on weighed paper and washing free from soluble proteid and chloride,

[1] Kühne: *Loc. cit.*, p. 363.

[2] Richards and Gies: *Loc. cit.*

[3] The amount of acid added was too slight to precipitate any mucin that may have been dissolved by the sodium chloride.

by drying at 110° C. and weighing. The following percentage results were obtained with ox ligament taken from as many animals:

	1	2	3	4	5	6	7	Average.
Fresh tissue.	0.565	0.429	0.539	0.510	0.490	0.574	0.569	0.525

Elastin. — Finely divided ox ligament from several animals, in quantities of 16 to 50 grams, after thorough extraction in 5 per cent sodium chloride solution was boiled in excess of water, with repeated renewal, until all collagenous fibres were removed by gelatinization and only very slight turbidity with tannic acid was obtainable in the cold concentrated filtrate. The undissolved residue was filtered on weighed papers, thoroughly washed free from traces of dissolved proteid and chloride, dried at 110° C. to constant weight and the percentage of elastin calculated from the weight obtained, with the following results:[1]

	1	2	3	4	Average.
Fresh tissue.	31.24	32.96	31.51	30.99	31.67

Collagen. — Eulenberg[2] observed long ago that ligamentum nuchæ yields gelatin on boiling. In these experiments the percentage content of collagen, in the form of gelatin, was determined gravimetrically. Weighed quantities, 20–40 grams, of finely divided fresh ox ligament were thoroughly extracted in half-saturated lime-water for several days at room temperature, for removal of albumin, globulin, mucin and extractives. Excess of calcium hydroxide was removed by washing in water. The tissue was then washed in alcohol and ether to remove fat, and finally boiled, in fresh portions of water, until only the merest turbidity could be obtained in small amounts of cold concentrated filtrate on addition of tannic acid. This process usually required six to ten hours. By this time all of the collagen was gelatinized and very little elastin hydrated. The filtrates were evaporated on the water bath in weighed crucibles, the residues dried at 100–110° C. to constant weight and gelatin determined, after subtraction of the ash obtained by burning the residue over a low flame, with the following percentage results:[3]

[1] This residue consists, strictly, of substances insoluble after such treatment. Only traces of non-elastin material could still be present, however — quantities too small to materially affect the results. Furthermore, a correspondingly small amount of elastin was probably lost by hydration.

[2] Eulenberg: See Schultze, Annalen der Chemie und Pharmacie, 1849, lxxi, p. 277.

[3] This method is, of course, open to the objection that possibly hydration pro-

	1	2	3	4	5	6	Average.
Fresh tissue.	7.61	6.77	7.38	6.99	7.13	7.52	7.23

Extractives. — Creatin and nuclein bases were detected qualitatively in aqueous extracts of large quantities of ligaments after removal of proteids and salts in the usual way, in confirmation of previous observations in this laboratory,[1] but no attempt was made to determine their quantity nor the character of the individual alloxuric bodies. In the summary below, extractives are included with the figures for "undetermined substance," which were obtained by difference.

Average composition. — The results of all our analyses are summarized in the following table, which gives the average percentage composition of fresh ligamentum nuchae and of the dry solid matter contained in it, and also the results of partial analysis of the ash:

Percentage composition.	Fresh ligament.		Dry ligament.		Ash.
	Calf.	Ox.	Calf.	Ox.	Ox.
Water.[2]	65.10	57.570			
Solids.	34.90	42.430			
Inorganic matter.	0.66	0.470	1.90	1.100	
SO_3.	. . .	0.026	. . .	0.062	5.64
P_2O_5.	. . .	0.035	. . .	0.081	7.39
Cl.	. . .	0.136	. . .	0.318	28.95
Organic matter.	34.24	41.960	98.10	98.900	
Fat (ether-soluble matter).	. . .	1.120	. . .	2.640	
Albumin, globulin.	. . .	0.616	. . .	1.452	
Mucin.	. . .	0.525	. . .	1.237	
Elastin.	. . .	31.670	. . .	74.641	
Collagen (gelatin).	. .	7.230	. . .	17.040	
Extractives and undetermined substance.	. . .	0.799	. . .	1.883	

ducts of the elastin increased the quantity of gelatin. In reality, however, such increase is insignificant when the hydration is carefully conducted and is probably

[1] RICHARDS and GIES: *Loc. cit.*

[2] The quantity of water in "elastic tissue" given, from BEAUNIS' Physiologie

just about equal in amount to the loss of gelatin in the removal tests with tannic acid. EWALD and KÜHNE (Jahresbericht der Thier-Chemie, 1877, p. 281) found that collagen is not digested by the proteolytic enzyme of pancreatic juice unless it has been previously swollen by acid or hot water, whereas most other proteids (including those we have found in the ligament), are digested without such preliminary treatment. We might have determined collagen directly by this process, perhaps, but we believe the one employed, a modification of HOPPE-SEYLER'S method (Handbuch der physiologisch- und pathologisch-chemischen Analyse, 1893, p. 482), gave results quite as accurate as could be obtained by the former or any other.

humaine, by HALLIBURTON (A Text-book of chemical physiology and pathology, 1891, p. 58) is 49.6%. The particular source of the tissue is not stated. This amount is lower than that for any of the connective tissues to which GORUP-BESANEZ referred (see page 287), and less than any others we have found recorded for particular forms of elastic tissue.

A NOTE ON THE CHEMICAL NATURE OF TRYPSIN.

By P. A. LEVENE.

[*From the Saranac Laboratory*, Dr. E. L. Trudeau, Director.]

THE object of my work was to inquire whether enzymes are actually of proteid nature as suggested very recently by Hans Friedenthal .The researches of Morochowetz, Lawrow, and Kutscher have shown that proteids can be digested by means of trypsin to such an extent that the product no longer gives the biuret test, in other words, the entire proteid material is decomposed. It has also been demonstrated by Gulewitsch that trypsin does not act on nitrogenous substances of non-proteid nature. It seemed to me, therefore, possible to test the proteid nature of enzymes by subjecting them to tryptic digestion.

Popoff also demonstrated that trypsin decomposes nucleoproteids, splitting off their phosphorus as phosphoric acid, and it seemed therefore possible to ascertain, by means of tryptic digestion, whether enzymes were of the same nature as nucleoproteids.

My first experiment was performed in September, 1899. Several pounds of fresh pancreas glands chopped fine, were treated with 0.5 per cent solution of sodium carbonate and a large quantity of chloroform and the mixture allowed to stand over night. It was then strained through gauze and the liquid was divided among several flasks, to which more chloroform was added. The flasks were placed in a thermostat at 40 C. Their contents were well shaken every day. After two weeks' digestion the contents of the flasks were filtered, the filtrate transferred into acid bottles, a considerable quantity of chloroform was added and the bottles were placed in a very warm room, in which they remained until May, 1900.

The solution, which was very dark in color, was then decolorized by means of animal charcoal, and tested for biuret. The result was negative. Another part of the same decolorized liquid was treated with a great excess of alcohol and the whole precipitate thus obtained tested for biuret without result. From these negative tests it was assumed that all the proteids of the original extract were decomposed. In order to test how far the decomposition of the nucleic acid went, a

determination of the phosphorus in the form of organic and inorganic compounds was made.

In 25 c.c. of the solution the phosphoric a id was precipitated by means of magnesia mixture. The slightly c olored precipitate was redissolved with hydrochloric acid and reprecipitated with ammonia. The $Mg_2P_2O_7$ weighed 0.257 gram.

Another 25 c.c. of the same liquid was evaporated to dryness and the residue fused with sodium carbonate and potassium nitrate. The phosphorus was estimated in the usual way. The $Mg_2P_2O_7$ weighed 0.259 gram.

This experiment demonstrated that trypsin is able to decompose absolutely the nucleocompounds of the pancreas, as well as the proteids. This solution of the self-digested pancreas extract, however, had no proteolytic activity.

It was then attempted to subject trypsin to self-digestion for a shorter period, so as either to break up all the nucleins and leave some proteid material intact, or vice versa.

Grubler's "trypsinum purissimum" was used for these experiments. About three grams of the substance was treated with 150 c.c. of 0.5 per cent solution of sodium carbonate and allowed to stand six weeks. At the end of that time the mixture was filtered. The filtrate gave a positive though very weak biuret test, and possessed tryptic activity. Fifty cubic centimetres of the solution was used for the estimation of the total phosphorus, and an equal portion for the estimation of phosphorus in form of phosphoric acid. The total phosphorus weighed 0.00125 gram and the phosphorus of the nucleocompounds weighed 0.0012 gram. Thus the absence of nucleocompounds in the solution was shown, and yet the solution contained the proteolytic enzyme.

In a second experiment, 30 grams of trypsin (Fairchild) was treated with 50 c.c. of 0.5 per cent solution of sodium carbonate (a great excess of chloroform being added as an antiseptic), and allowed to stand in an incubator. After three weeks of self-digestion part of the mixture was filtered, and tested for proteolytic activity. The result was positive. In 25 c.c. of the filtrate, the total phosphorus weighed 0.0162 gram. In another 25 c.c. portion the phosphorus of the mineral phosphates weighed 0.0131 gram. Traces of nucleocompounds were thus shown to be present.

After four weeks of self-digestion the solution still possessed its proteolytic properties. In 10 c.c. 0.00715 gram of phosphorus was

found. Another 10 c.c. of the same solution contained 0.00628 gram of phosphorus as phosphates. This again showed the presence of a slight amount of nucleocompounds.

After six weeks of self-digestion the solution was still active and gave the biuret reaction. In 10 c.c. of the solution the total phosphorus weighed 0.00715 gram. In 10 c.c. of the same solution the phosphorus as mineral phosphates weighed 0.00663 gram. Thus the solution contained scarcely any nucleocompounds and yet possessed proteolytic activity.

These experiments would scarcely justify the conclusions of Friedenthal that trypsin is a nucleocompound.

The fact that only those solutions were active which gave a positive biuret test would seem to indicate that trypsin is of proteid nature. However, in some cases the biuret test was scarcely perceptible, and yet the solution of self-digested trypsin still contained the active ferment.

Similar experiments on other enzymes are now in progress.

I wish to express my indebtedness to Dr. D. Sculley for his kind assistance.

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STUDIES ON THE EFFECTS OF ELECTRICITY ON ORGANISMS.[1] II. — THE REACTIONS OF HYDRA TO THE CONSTANT CURRENT.[2]

By RAYMOND PEARL.

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I. Introduction.

IN the course of some investigations now in progress, on the general question of the physical and chemical effects of electricity on organisms, it happened that some experiments were tried upon the common Hydra. It was at once noted that the reactions of the animal to the current were of a peculiar character, and as they seemed to present some points of general interest, a detailed study of the phenomena was made. The results here presented are the outcome of this study. The points considered at this time have to do entirely

[1] This paper is the second of a series on the general subject of the effects of the electric current on protoplasm and organisms. The first number of the series was entitled: "Studies on Electrotaxis. I. — On the Reactions of Certain Infusoria to the Electric Current," and was published in this journal, 1900, iv, pp. 96–123. It has seemed best to change the main title in order to more satisfactorily indicate the scope of the work.

[2] Work from the Zoölogical Laboratory of the University of Michigan, Jacob Reighard, director.

with the general responses of the animal to the current, the disintegration phenomena being reserved for discussion in a later paper.

It is well known that Hydra is able to move about from place to place under certain circumstances; but it is during the greater part of the time attached at one point and is for all practical purposes a sessile form. The movements of the body while the Hydra is attached fall into two main categories: (a) sudden, general contractions of the whole body, and (b) bending movements of the extended body. Besides these body movements there are, of course, bendings and contractions of the tentacles. The bending movements of the body are the result of active muscular contractions on one side. So far as has been known Hydra shows no precise orientation in response to any stimulus. The nearest approach to an orienting reaction is found in its phototaxis which has been described by Wilson,[1] but in this case the movement toward the light is described as a more or less irregular wandering. There is no evidence of a precise orientation to light in the case of an attached Hydra. The aim of the present study was to determine whether an attached Hydra orients itself to the constant electric current, and, if so, what the mechanism is by which this orientation is effected. It was found that a definite orientation does occur.

I have been able to find in the literature of this subject only two references relating to the effect of the current on Hydra. The first of these is in a paper by Zoja,[2] in which a section is devoted to the effect of the induced current on different species and under different conditions. The results were obtained by placing the electrodes in contact with the body, and no mention is made of anything like an orienting reaction. The contribution is not important from our standpoint. The other reference is a brief note in a valuable paper by Roux.[3] He describes a polar disintegration of the cells at the surface produced by the action of an alternating current on Hydra fusca.

We will now pass to a detailed consideration of the experiments.[4]

[1] Wilson, E. B.: American naturalist, 1891, xxv, pp. 413–433.

[2] Zoja, R.: Bollettino scientifico, 1890, xii, pp. (of separate) 1–90. An abstract of this paper was printed in the Archives italiennes de biologie, 1891, xv, pp. 125–128.

[3] Roux, W.: Sitzungsberichte der kaiserlichen Akademie der Wissenschaften zu Wien. Mathem.-naturw. Classe, 1892, ci, Abth. iii, pp. 1–208.

[4] It is a pleasure to acknowledge my indebtedness to Dr. H. S. Jennings for suggestions freely given during the course of the work, and for much valuable criticism.

II. Material and Methods.

The form most used was the common green Hydra, Hydra viridis, which was abundant in cultures of Ceratophyllum in the laboratory. Hydra fusca was used to some extent at the beginning of the work; but on account of its comparative scarcity and the fact that its reactions were in all essentials the same as those of the green Hydra, it was abandoned in favor of the latter. The apparatus for obtaining the current was the same as that which I have fully described in another place,[1] so that a mere mention of it will suffice here. The current was taken from the lighting circuit, reduced to the proper intensity by interposed resistance, and led to the preparation by means of unpolarizable brush electrodes. A rheostat was used for varying the intensity.

I found the most satisfactory method of observation was to place the Hydra on a slide in a drop of water under a square cover glass supported at the two opposite ends by rectangles of filter paper one or two layers in thickness. These were wet with culture water. On the projecting ends of the filter papers were laid the brushes of the electrodes. The animals became fully extended in this moderately thin layer of water and study with high powers was possible. This mode of examination was controlled by a study of the animals in a trough containing a sufficient depth of water to admit of free movement in all directions.

III. Observations.

As will appear later, the method of reaction taken by Hydra depends on its initial position with reference to the direction of the current. For this reason the description will be divided according to the different *initial positions of the animal.*

Reactions of Hydra when at right angles to the direction of the current. — Suppose a Hydra, attached by the foot to the slide, to be fully extended under the cover glass with the long axis of its body at right angles to the direction of flow of the current. This will of course bring some of the tentacles in line with the current and others at right angles to it. If now a very weak current be made the immediate result will be a contraction of those tentacles which are extended parallel to the direction of the current and which lie on the

[1] Pearl: This journal, 1900, iv, p. 98.

cathode side of the body. Simultaneously with, or shortly after the contraction of the tentacles on the cathode side, those on the anode side which are in line with the current as a rule also contract, but not so vigorously as the former. This tentacle contraction consists merely in a shortening of the tentacle without any change in its direction. The amount of contraction depends on the strength of the current; but even in very weak intensities the cathode tentacles usually contract very strongly. The contraction is rapid and usually occurs immediately after the circuit is closed. The contraction of the anode tentacles is considerably less in amount than that of the tentacles on the cathode side, and often does not occur at all. The tentacles which lie at right angles to the direction of flow are not affected in any way by a weak current. They remain extended while the current is passing, provided there is no general contraction of the whole animal. This special reaction of the tentacles in weak currents has a certain resemblance to the well-known phenomena shown by the pseudopodia of Actinosphaerium under the action of the same stimulus.

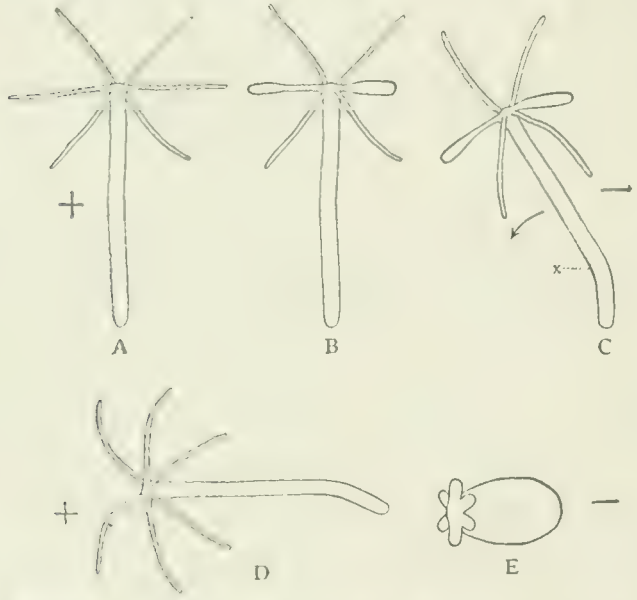


FIGURE 1. — Diagram showing the orientation of Hydra according to "Reaction I." A, position at moment of making the current. B to E, successive phases of the reaction. In all the figures contracted regions are indicated by heavy lines.

At the same time that the tentacles are showing the phenomena above described, or a little later, the body of the Hydra begins to bend very slowly towards the anode (Fig. 1, C). In a typical case this bending usually occurs mainly at a point a short distance above the foot (Fig. 1, C, x), although it may include the whole length of the body. It is evidently a result of the contraction of the muscle cells on the anode side in the region indicated. The bending is somewhat slow and is not always a continuous movement, but may be interrupted by frequent stops. This bending is often accompanied by a twisting of the body on its long axis about the foot as a fixed point. The twisting is not antagonistic to the bending, but works

with it, tending to bring the animal sooner into line with the current. There may also often be observed a secondary contraction of the body on the anode side at a point about as far below the hypostome as the point x (Fig. 1, C) is above the foot. This secondary bend of course results in orienting the head end in line with the current while the rest of the body is still nearly transverse to the current direction, as shown in Fig. 2.

The bending toward the anode continues till the long axis of the body is very nearly or quite parallel with the direction of flow of the current, or in other words, until orientation is complete (Fig. 1, D). While the main part of the body is swinging about x (Fig. 1, C) as a fixed point the foot itself turns at its point of attachment, in the same direction, till the end result is orientation in the line of the current with the whole body straight.

Usually immediately after the animal becomes oriented a partial or complete contraction of the whole body occurs (Fig. 1 E). After this contraction the animal may become extended again and remain so, or it may stay contracted for a long time. The most usual behavior after orientation is a succession of extensions and contractions at intervals of less than a minute.

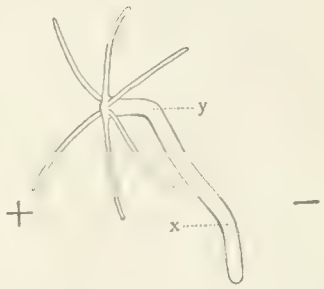


FIGURE 2. — Diagram showing secondary anode contraction (y) of Hydra in transverse position.

As the bending into orientation occurs, the tentacles of course change their axial relations to the direction of the current. As this change occurs the cathode and anode contraction phenomena disappear and the tentacles become more or less extended and remain so. After orientation is complete all the tentacles become more or less turned back over the oral end of the body, so as to point toward the cathode, as indicated in Fig. 1, D.

The bending of the body into line with the current in the reaction just described we may call the *orienting response* in distinction from the general contraction reaction[1] of Hydra. The orientation in the typical case which has been considered, is produced by a bending of the body without any intervening general contraction of the animal as a whole. Exactly this form of response occurs only in very weak currents.

[1] By "general contraction reaction" is meant the violent contraction of the whole body which occurs when the animal is strongly stimulated in any way.

The deviations in form of reaction from this typical case are numerous, but for the most part they fall under two main classes, which may be distinguished as follows: (a) those reactions in which a general contraction response appears before, or during the early part of the orienting process, and (b) the reactions in which the foot becomes oriented before the remainder of the body. For the sake of verbal economy and convenience we may refer to the typical case which has been described above, as "Reaction I."

We will now consider in some detail the two classes of variations from the typical reaction.

The first of these methods of reaction usually takes place in slightly stronger currents although in the case of many individuals it is the only form of special reaction which occurs in even the weakest effective currents. In it there is the same sort of an orientation as in the typical case, but it is produced in a different way. When the animal is extended at right angles to the current as before, immediately on closing the circuit it violently contracts (Fig. 3, B). In other words it gives the "general reaction," as distinct from the "special orienting reaction." While contracted it turns quite rapidly about the foot as a fixed point, through the position shown in Fig. 3, C, until it becomes oriented (Fig. 3, D). It then extends and its subsequent reactions are like those described above for the typical case. This method orients the animal faster than any other, in many cases the time intervening between the making of the current and complete orientation not being more than twenty to thirty seconds. It is apparently, like Reaction I, due to a contraction on the anode side, but in this case the special orienting contraction is all at the foot and is more rapid than in the former reaction. There is no special reaction of the tentacles as in Reaction I, since they contract suddenly and completely at the same time with the body. This method of response may be referred to as "Reaction II."

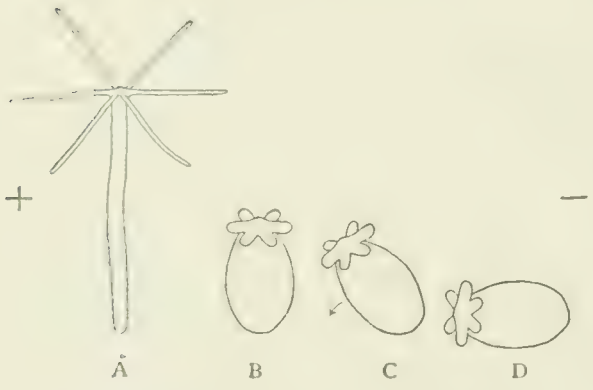


FIGURE 3.—Diagram showing orientation by "Reaction II." Letters as in Fig. 1.

The second important case of variation from the typical method

of reaction is in a sense a combination of the methods which have been described. At the time of closing the circuit there is no general contraction, but the foot end of the body, from the point of attachment to about the point x (Fig. 4, B), begins to turn rapidly toward the anode, just as the whole body does in Reaction II. This rapid swinging of the foot while the rest of the body remains more or less nearly in its original position, gives the animal as a whole a curve with its concavity toward the cathode (Fig. 4, B). Usually after the foot has turned in this way till it is nearly in orientation, the animal gives the general contraction reaction (Fig. 4, C), and then the contracted body swings into orientation as in Reaction II (Fig. 4, D). Before the general contraction reaction occurs the secondary anode bending at the head end as described for the typical reaction may appear. The tentacles show the same special reactions up to the time of the general contraction of the body as were described for an individual giving the typical reaction.

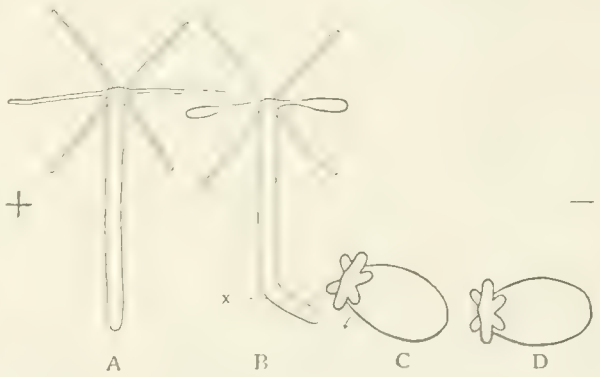


FIGURE 4. — Diagram showing orientation according to "Reaction III." Letters as in Fig. 1.

This reaction is evidently of somewhat the same character as Reaction II, except that the general contraction does not immediately follow the making of the current. The essential difference of this from all other methods of reaction is found in the fact that the turning which results in orientation is primarily of the foot region alone and is about the point of attachment as a centre. When the sudden general contraction occurs, the animal straightens in such a way that its long axis forms the same angle with the direction of the current as that which the foot region had previously attained. In other words the contraction straightens the curve in the body without changing the position of the foot region. This variation from the typical response will be referred to as "Reaction III."

The three general types of reaction above described include the methods most usually taken by Hydra in reacting to weak currents. It is apparent that the three types have one point in common, namely that orientation with the oral end toward the anode is produced by a local contraction on the anode side of the body. They may

be considered as variants from a single reaction. Reactions I and III seem to depend upon something different in the organization of the individuals exhibiting them. They both take place in the same strength of current, some individuals showing Reaction I, and others Reaction III. On the other hand, Reaction II seems to be a response depending on the action of a current greater in intensity than the weakest effective current. The same Hydra may in the weakest effective current orient by Reaction I, and, in a stronger current, by Reaction II. The reason for distinguishing Reaction II is the fact that in the case of some individuals it is impossible to so regulate the current as to produce an orienting response of any other sort.

The three general forms of reaction which I have described seem to depend in a certain way on the strength of the current, but the particular current which produces them varies with different individuals. The closeness of the relation of the form of reaction to these

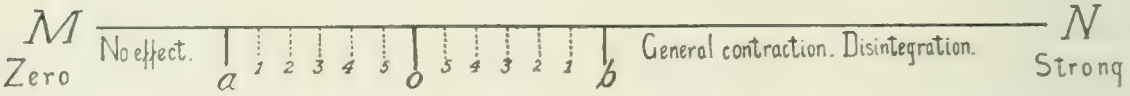


FIGURE 5. — Diagram showing the relation of the reactions to the current intensity.

two factors, the individual and the current intensity, is shown by the fact that in some individuals it is impossible to produce a special orienting reaction by the most delicate gradation of the current. A current strong enough to be effective results in sudden, violent and continuous contraction without orientation as in Reaction II. There is a certain range of current intensity within which orientation occurs by one type or another of reaction, but this range varies very much among individuals. This may be shown graphically as in Fig. 5, in which the line *M N* represents the whole range of current intensity, and the portion *a b* represents that range within which orientation occurs. For different individuals the end values *a* and *b* may approach each other as indicated by the dotted lines, 1, 2, 3, 4 and 5, till in some cases they may coincide at *o* and then that individual will not show any special orienting reaction.

Of minor importance are the variations from the types. These are due to spontaneous and secondary contractions of the body occurring at the same time as the special orienting reaction. Such variations are of very wide range and universal occurrence, or, expressed in another way, "no two individuals react to the current in precisely

the same way." The essential fact, however, of contraction on the anode side occurs in practically all cases.

Special reactions of unattached Hydras. — If at the time of making the current the foot end is not attached and if as before the animal is at right angles to the direction of the current, essentially the same phenomena occur as in the cases just described. There is a contraction on the anode side, but in the unattached animal this usually results in the production of the form shown in Fig. 6, B. The body forms a bow with the concavity towards the anode. If both the head and foot ends are free to move no further reaction takes place. No orientation occurs. If however, either the oral or the foot end becomes fixed, or even hindered in its movement, as for instance by striking a bit of debris, then an orientation or an approach to orientation occurs. If it is the foot end which becomes hindered in its movement, orientation is produced in essentially the same way as in Reaction I of an attached individual. It frequently happens that the foot attachment of a Hydra becomes loosened and the tentacles become fastened to the substrate in the way which has been described by Zykoff.[1] Such an animal reacts to the current by contracting on the anode side, but in this case, the oral end being fixed, the rest of the body swings about that as a centre. The necessary mechanical result of this is that the animal becomes oriented with the oral end toward the cathode. The series of changes observed in this process in some cases is shown in Fig. 7. Orientation in this way is a slow process, as before the complete orientation of the whole body is attained the oral end must change its position. This is accomplished by the movements of the tentacles which change their positions and points of attachment to the slide. At the same time, there is frequently observed a twisting of the body near the head, which assists in the process. After the animal gets into line with the current it stays in the same position quite as well as though the head were directed toward the anode.

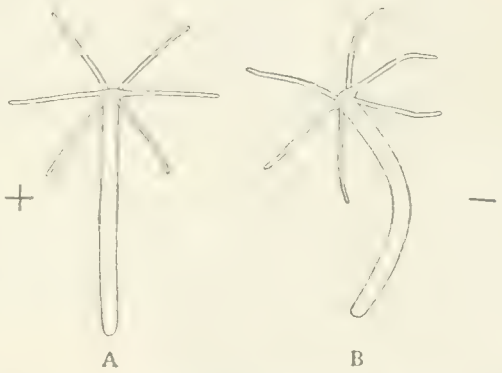


FIGURE 6. — Diagram showing the reaction of Hydra when the foot is not attached.

[1] ZYKOFF, W.: Biologisches Centralblatt, 1898, xviii, pp. 270-272.

General contraction reaction. — If a current of more than a certain intensity be passed through a Hydra in the transverse position there results an immediate, strong, general contraction of the whole body including the tentacles (Fig. 3, B). This is the same sort of a reaction as is produced by stimulating the animal mechanically or in any way suddenly changing the environmental conditions. As has been pointed out in the discussion of the special reactions this general contraction may precede, or occur at various stages during the process of orientation. In strong currents, however, orientation does not usually follow the general contraction reaction. The animal having strongly contracted remains in that condition, without further change, till death ensues. This seems to mean merely that the strong stimulus calls forth such a vigorous general response that there is no possibility of further polar orienting contraction of the already maximally responding contractile elements. As in the case of the special orienting reaction the minimal current intensity which will produce the general contraction varies greatly with different individuals. Of course, in case of the general reaction there is no upper limit of current intensity beyond which the response is no longer produced, as, the stronger the current, the stronger the contraction produced.

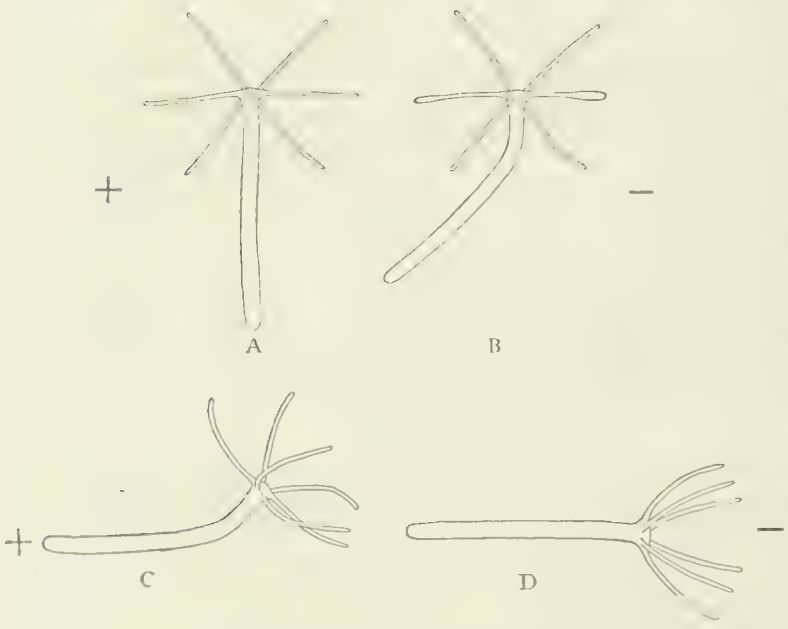


FIGURE 7. — Diagram showing the method of orientation of a Hydra with the foot unattached.

The general contraction reaction follows primarily a sudden change in environmental conditions. This can be very well shown by the use of the current, for, if the current be very gradually raised in intensity from zero, by slowly turning the rheostat, the intensity at which the general contraction appears is considerably greater than if the circuit had been suddenly closed. In other words, a gradually increased

stimulus does not cause the general reaction until it is far past the usual minimal intensity for that reaction. I wish, however, to emphasize the fact that regardless of how gradually the intensity of the current may be increased the general contraction eventually results in all cases. It may be noted at this point that the special orienting reaction for any given individual begins at a certain intensity of the current whether that intensity is reached by gradual steps or by a sudden closing of the circuit.

We shall next consider the reactions of animals in other positions with reference to the direction of the current, and in closing this section it may be well to sum up the observations in a single statement. An attached Hydra extended transversely to the direction of the current at the time of making reacts, provided the current is weak, by a local contraction on the anode side of the body ; and, the foot remaining fixed, this contraction results in orientation with the oral end toward the anode. Those tentacles extended parallel with the current contract. An unattached Hydra, lying transverse to the current, reacts in the same way by a contraction on the anode side. The result of this contraction in unattached animals is different under different circumstances. In currents above a certain intensity, the only reaction is a sudden, strong general contraction of the whole body.

Reaction of Hydra when the long axis of the body is parallel to the direction of the current. — When the long axis of the body is parallel to the direction of the current, the precise nature of the reaction depends on the position of the ends of the animal with reference to the electrodes.

When the oral end is toward the cathode the making of a current of weak or medium intensity produces no immediate reaction. The animal remains extended and quiet for some time, and then usually gives the sudden, general contraction response. In some cases the time intervening between the making of the current and the contraction was so great that it was impossible to tell whether the reaction was induced by the current or was merely one of the general contractions which occur at frequent and somewhat regular intervals in an unstimulated animal. Usually, however, the contractions occurred from thirty seconds to a minute after the closing of the circuit. There appears to be no evidence of a polar reaction in this case. After remaining contracted for a short time the body extends again, whether the current continue to pass or not.

When the oral end is toward the anode at the time of making the current the reaction is of the same form, *i. e.*, the general contraction; but the contraction occurs in almost every case immediately, in any strength of current which is effective. In very sluggish animals and in those which had been for some time under the influence of the current there was sometimes an interval of from three to six seconds between the making of a very weak current and the contraction. In medium and strong currents the reaction was always immediate. Complete extension does not occur at once after the contraction. The animal begins extending, but is immediately stimulated to another contraction, so that often the result is a sort of rhythmical shortening and lengthening of the body. This reaction is the same as has already been described for animals which have attained orientation from a transverse position.

The animal shows no tendency to turn into orientation with the oral end toward the anode when this end is toward the cathode. It simply remains in that position. This is in no way different from what would be expected, since orientation is brought about by contraction on the anode side, and in this case there is no anode side but an anode end of the body; and, as has been stated above, there is no observable polar difference of contraction when the animal is in line with the current.

There is evidently only one form of reaction when the long axis of the body is parallel to the direction of the current, *i. e.*, the general contraction response. The only difference between the effect when the oral end is toward the anode and when it is toward the cathode, is that in the former case the reaction does not occur for a considerable time after the current has been passing, while in the latter case the contraction is immediate. The only trace of a special reaction was found in the fact that the tentacles in very weak currents have a tendency to show contraction on the cathode side. This results in a different position of the tentacles according as the oral end is toward the anode or the cathode. When the oral end is toward the anode the tentacles are bent back over the body (Fig. 1, D), while with the oral end toward the cathode they stretch out in front (Fig. 7, D). It will readily be seen that these results are due to a contraction of the tentacles on the cathode side. This special reaction of the tentacles occurs only in currents too weak to cause the violent general contraction and in some individuals it does not appear at all, evidently because the individual is not "attuned" in sensitivity to the current

in such a way as to make the reaction possible. The same phenomenon is usually seen after an animal attains orientation in the ordinary way provided the tentacles are not completely contracted.

Reactions of separated pieces of the body. — A series of experiments was made using parts of the body of Hydra separated by transverse cuts. In most cases the animal was divided into nearly equal halves.

An oral piece shows the same special reactions of the tentacles and body as are given by a whole animal. The tentacles in line with the current contract, those on the cathode side showing a stronger contraction, and reacting in weaker currents, than those nearer the anode. The body shows contraction on the anode side. This does not usually produce an orientation, but I have observed that in some cases oral pieces become oriented with the oral end toward the cathode as has already been described for a normal animal with the foot unattached. The anode contraction of an oral piece is usually more or less localized at the point just behind the tentacles where the secondary contraction of a complete individual occurs (Fig. 2, y). The reactions of an oral piece extended with the long axis in line with the current are the same as those of a complete animal in the same position. Such pieces do not appear to be either more or less sensitive than a normal complete animal.

In the aboral half of the animal orientation from a transverse position by a contraction on the anode side near the foot, occurred just as in a normal animal. The reactions of such a piece in line with the current direction were essentially the same as in the normal animal in a similar position.

The body of Hydra, so far as its reactions to the current go, seems to form a sort of physiological "equi-potential system." Or, in other words, the body of the animal, leaving out of consideration the tentacles, is not structurally differentiated into parts which are so differently affected by the current as to modify the reaction of the whole, provided they are by operation brought into prominence. The neuromuscular mechanism of Hydra, to the stimulation of which the orienting and general contraction responses are probably due, is made up of elements essentially alike over all parts of the body, and there is no central controlling factor like an organized central nervous system, so that when any part of the body is put under the action of the current we have the same mechanism acted upon by the same stimulus and the same reaction is the result. It is to be understood that reference is made merely to the contraction and orienting phe-

nomena and not to the disintegration, which, being a purely physical affair, is mechanically conditioned in its form by the portion of the body which is disintegrating.

Reactions of buds. — From the experiments made upon Hydras bearing buds it was evident that, after the bud has attained a considerable degree of development, parent and bud are independent in their reactions. This fact has been noted by Zoja.[1] The independence is very well shown by placing an animal in the position shown in Fig. 8. The arrangement is such that the oral end of the parent animal is toward the cathode, in which position, as has been shown, there is no immediate contraction, while the bud occupies a transverse position. It is then possible by the use of a weak current to obtain an approximate orientation of the bud without affecting the parent in any way. The bud reacts as an independent animal in that it contracts on the anode side at a point just above its place of attachment to the parent and exhibits reactions of the tentacles like those of an adult. The bud is much more sensitive than the adult to stimulation by the current, and for this reason I have not been able to produce any orientation of the parent without producing a violent general contraction of the bud.

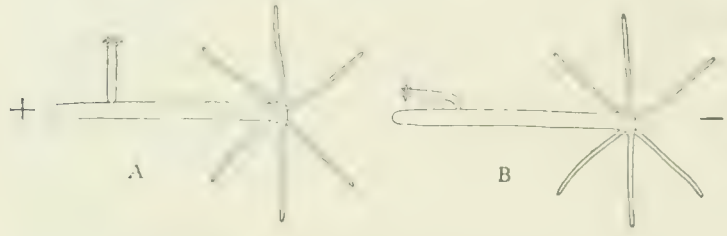


FIGURE 8. — Diagram showing the reaction of a bud.

When the bud is extended with the long axis in line with the current at the time of making, in any except the very weakest currents, contraction occurs immediately, regardless of whether the head is toward the anode or the cathode. This is probably a result of the great sensitiveness of the bud, because it has been possible to produce a general contraction of a bud with its oral end toward the cathode, in currents so weak as not to affect in any way the sensitive tentacles of the parent animal.

It then appears that a bud almost ready to become detached from the parent[2] exhibits the same phenomena of general contraction and orienting reactions as would be shown by an adult individual under the same conditions with reference to the direction of the current. The only striking difference in the two cases is that the bud is more sensitive than the adult.

[1] ZOJA: *l. c.*, p. 55.

[2] I have not been able to test the effect of the current on very young buds, on account of a lack of material in the proper stage of development.

The effect of very strong currents. — In a very strong current which practically immediately kills the animal very characteristic phenomena appear. There is first an immediate general contraction of the whole body. Following this, or occurring at the same time, in case the long axis of the body is at right angles to the current, there is a single sharp secondary contraction on the *cathode* side. This swings the animal slightly toward the cathode (Fig. 9). This is evidently not due to any passive carrying by the current but represents an actual vigorous contraction, because in the first place it is in the opposite sense from that in which the cataphoric action would tend to carry it and from that in which the cataphoric action actually does move dead Hydras in some cases. Furthermore it is a very sudden, jerky movement, different in character from that produced by the purely physical action of the current. After this cathode movement has taken place the granular disintegration of the body immediately begins.

In case the long axis of the body is in line with the current at the moment of making such a strong current, the general contraction occurs and is immediately followed by the beginning of the disintegration. There is no secondary contraction as before.

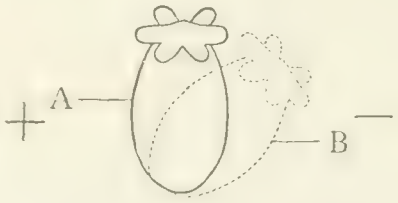


FIGURE 9. — Diagram showing the cathode contraction in a very strong current.

The disintegration phenomena embrace two phases, one, the effect on the ectoderm, and the other, the disintegration of the entoderm. The changes in the ectoderm, as has already been noted by Roux,[1] consist in a swelling of the cells and a partial emptying of their contents. The cell boundaries become invisible. The entoderm cells undergo almost complete disintegration and their contents are discharged at the oral end of the body (except in rare cases), regardless of the position of the animal with reference to the current direction. I shall not further discuss this matter of disintegration at the present time, but hope in a future paper to describe the phenomenon more fully and show its relation to the general problem of the effect of electricity on protoplasm.

Current intensity relations. — In the descriptions up to this point use has been made of the terms "weak," "medium," and "strong" in designating the intensity of the currents used. The reason for not giving more precise statements of the absolute strength of the current

[1] ROUX: *Loc. cit.*, p. 62.

is to be found in the fact that has already been brought out, namely, that there is a large amount of variation among different individuals in the way they are affected by the current. As close measurements as possible of the current have been made and recorded in many experiments, but their introduction into an account of the phenomena would mean the addition of a mass of detail which would really be of very little value to one wishing to repeat the experiments. The most useful method of giving an expression of the intensity relations is by defining in terms of δ the limiting values of the words "weak," "medium," and "strong" as used, and furthermore by making comparisons with some common form like Paramecium which shows well defined reactions to the current. This will enable any one, having fulfilled the conditions necessary to obtaining the reaction of Paramecium, to repeat experiments on other organisms.

The limiting values of the terms "weak," "medium," and "strong" as here used are as follows: "weak," intensities of less than 8 δ; "medium," intensities between 8 δ and 20 δ, and "strong," intensities of over 20 δ.

In comparing the reactions of Hydra with those of Paramecium with reference to current intensity it may be stated as a general rule that Hydra viridis is more sensitive to the current than is Paramecium. Under the same conditions the orientation of Hydra after the type of "Reaction I" occurs in currents of from one half to three fourths the intensity necessary to produce a well-marked movement of Paramecia toward the cathode. Correspondingly, the intensity at which disintegration phenomena become apparent in case of Hydra is lower, by about the same amount, than in Paramecium.

For reasons which have been stated elsewhere (p. 308) in applying the current to Hydra it is absolutely necessary to have some means whereby very *fine gradations* in the current intensity may be obtained.

The effects of breaking and reversing the current. — In no position of the animal have I been able to detect any "break shock" when the circuit was opened. The animal simply renews its normal activities after the cessation of the action of the current. The sudden change of intensity in environmental conditions from strong stimulation to normal conditions in this case does not cause any sharp reaction.

Reversal of the direction of the current usually causes the sudden, general contraction reaction. The only exceptions to this are cases of two sorts: first, those in which a very weak current is acting on a

somewhat resistant animal lying with the oral end toward the anode. In such cases the reversal (bringing the oral end toward the cathode) does not in most instances cause the contraction. The second class of cases includes those animals which are again not extremely sensitive, and are in a more or less transverse position acquiring orientation. Often under such conditions the only effect of reversal is to start the animal contracting on the new anode side, without any intervening general contraction.

IV. Discussion of Results.

In discussing the results gained from this study it seems proper to examine first the relation between the reactions of Hydra to the current, and its reactions to other stimuli.

It is evident from the observations recorded that Hydra shows two distinct sorts of reaction to the current. One of these is a generalized, violent contraction of the animal as a whole, and the other is an orienting reaction. These two forms of response may be considered more in detail.

The general contraction reaction which the animal gives in currents above a certain intensity is, so far as can be determined, in no way different from the response to a strong mechanical or chemical stimulus. It is in a way a "reflex" of the organism, which occurs whenever there is a more or less *sudden* change in environmental conditions. The character of the environmental change makes no difference in the character of the response. This reaction to the current is psychologically the same as that in response to any other more or less violent stimulus. It is the start which the animal gives when unduly stimulated. It may be compared with the start of a human being on receiving a shock from an electrical machine or an induction coil. The start or "motor reflex" of a person under such circumstances is not essentially different from his reaction when suddenly struck, or dashed with cold water. In other words the reaction of the man is not due to the distinctive physical properties of the current as such.

The second of the reactions of Hydra to the current, the orienting response, is essentially a slow bending of the body, taking place in such a way as to bring the long axis into line with the current. It is not due to a *sudden* change in the environment but can be called forth by a very gradual increase in the strength of the current as well

as by suddenly making a current of the requisite intensity. In this reaction the Hydra is apparently only partially stimulated. Or, in other words, when the animal performs the orienting reaction it is not stimulated as a whole, a psychological individual, as is the case when it gives the general contraction response. A part of the muscular mechanism (that on the anode side in the case of the body) is set into activity without stimulating the whole animal. To pursue the comparison with the reactions of a human being to the current, we may consider this orienting response to correspond to the excessive and purely involuntary contraction of the muscles of the hands grasping the electrodes under the action of a strong current. The strong gripping of the electrodes is in no sense a psychological reaction of the man, necessarily depending upon his being stimulated as a whole.

A comparison may be made to a certain extent between the two sorts of reaction to the current in the case of Hydra, and what I have found for the infusoria (*loc. cit.*). We may consider the general contraction response of Hydra as corresponding to the "motor reflex" factor in the reactions of the infusoria. The point in common is that both reactions are given in response to a variety of stimuli and are not effects peculiar to the action of the current. The orienting reaction of Hydra may be compared to the "forced movement" factor in the electrotaxis of the infusoria. The point in common is that both are due to local effects bearing definite relations to the poles of the electric field. In the case of the infusoria this local effect is expressed in the reversal of the cilia on the cathode side, while in Hydra the expression is found in the contraction of the body on the anode side. The evidence now at hand does not warrant the conclusion that these two factors in the case of Hydra and of the infusoria are physiologically the same, but on the other hand, there are some important features common to both.

A point of some theoretical interest in connection with the orienting phenomena in the case of Hydra, is the fact that the orienting process brings the animal into that position where it is most strongly stimulated, *i. e.*, with the oral end toward the anode. As was shown in the section on the reaction when the longitudinal axis is in line with the current direction, the organism is least stimulated when the oral end is toward the cathode, and most stimulated in the opposite position. The orienting process is, however, always of the same kind and brings the oral end toward the anode or cathode

according as the animal is, or is not, attached by the foot. The orientation is as pronounced and permanent in one case as in the other. This indicates that the essential thing in the orienting process is not the getting of the organism into a position where it is not stimulated or only slightly stimulated, but that, on the contrary the orientation takes place without any reference to whether the animal is to be stimulated in its end position or not. This point has been extensively developed by Loeb in connection with the heliotropic and other orientations. Furthermore it appears that the placing of one or the other end of the body toward the source of the stimulus is not the essential of the orientation, but is rather a result of the mechanical relation of the organism to surrounding objects, while the important thing is the establishment of definite relations of the axes of the body to the lines of action of the directive stimulus. That the placing of one or the other end of the body toward the source of stimulus is not the essential element in the orienting response to light, in some cases at least, has been well brought out by Cole[1] in his work on the phototaxis of pycnogonids. He shows that an individual pycnogonid moves toward the light with either the anterior or posterior end in advance according as it is crawling or swimming, the reaction of the animal being precisely the same in the two cases, while the result is due to the mechanical relations of the organism to the bottom. It seems possible that it may be found to be a general rule that in orientation phenomena the position of the ends of the body with reference to the source of the stimulus is secondary, and that the primary factor is the relation of the longitudinal axis to the lines of action of the stimulus.

V. Summary.

1. Hydra viridis, when attached by the foot, becomes oriented from a transverse position in constant currents of weak intensities, so that the long axis of the body is in line with the current and the oral end is toward the anode.

2. This orientation is brought about by a contraction on the anode side of the body.

3. When the Hydra is not attached by the foot it may become oriented, as a result of a contraction on the anode side, with the oral end toward the *cathode*.

[1] Cole, L. J.: Biological bulletin, 1901, ii, pp. 195–207.

4. In addition to the orienting reaction to the current, there is a general contraction response.

5. When Hydra is in line with the current it reacts by a general contraction, being apparently more strongly stimulated when the oral end is toward the anode.

6. The tentacles show contraction phenomena on the cathode side.

7. Separated pieces of the body react in essentially the same way as whole individuals.

8. Buds and parent animals are independent in their reactions. The buds show essentially the same reactions as adults.

ON THE OXIDATION OF NATIVE PIGMENTS.

By WALTER JONES and JOHN AUER.

[*From the Laboratory of Physiological Chemistry in the Johns Hopkins University.*]

IT has already been shown [1] that when the black pigment of horse-hair is treated in turn with concentrated hydrochloric acid and melted potassium hydroxide a substance survives which, aside from its freedom from sulphur, is characterized by all of the properties which we are accustomed to ascribe to the melaninic acids. As it is scarcely conceivable that the material obtained by such a process can be contaminated by any constituent of the tissue in which it is found, or at least by any well known constituent, the substance should serve as excellent material for an investigation of the decomposition products of the pigment; for in case a decomposition product is obtained, it cannot be attributed to the decomposition of adherent proteids, but must be referred to the pigment itself.

This is especially important, since, through the researches of Schmiedeberg,[2] Nencki,[3] Chittenden and Albro,[4] and others, the melanins have come to be regarded as derivatives of proteids; and we might expect to find closely related if not identical decomposition products in the case of both classes of compounds.

The method by which the material for this work was prepared does not differ essentially from that described in our former paper. Special precautions, however, were observed in order to insure the freedom of the pigment from any trace of fat or fatty acid; and as we had reason to suspect that previous failures to find decomposition products were due in some measure to the use of an insufficient quantity of material, an estimation of the carbon was made in a measured portion of the pigment emulsion. Data were thus obtained

[1] JONES: This journal, 1899, ii, p. 380.

[2] SCHMIEDEBERG: Archiv für experimentelle Pathologie und Pharmakologie. 1897, xxxix, p. 1.

[3] NENCKI: Berichte der deutschen chemischen Gesellschaft, 1895, xxviii, p. 560.

[4] CHITTENDEN and ALBRO: This journal, 1899, ii, p. 291.

which showed how much pigment was involved in any one experiment and which served as a basis for the quantitative estimations that will be described.

The Method of Oxidation.

A comparatively large portion of melaninic acid was alternately dissolved in dilute caustic soda and reprecipitated with a small amount of very dilute hydrochloric acid until the wash liquid contained only a small quantity of salt and a very slight excess of acid. After the last precipitation the material was allowed to settle several days, the liquid was closely siphoned off and a portion of 40 c.c. of the well mixed pigment mud was placed on a weighed filter paper and allowed to drain until the pigment had scaled off the paper. The pigment was then dried to a constant weight at 105 degrees, and the part that could easily be removed from the paper was ground in an agate mortar. A weighed portion of the material was submitted to an elementary organic analysis and by an obvious calculation it was found that one cubic centimetre of the emulsion contained a quantity of melaninic acid that corresponds to 0.00195 gram of carbon.

A portion of the same pigment mass was treated with a very dilute solution of caustic soda in small successive portions and from time to time a drop of the material was removed and tested with litmus. The reaction of the fluid which was in the beginning acid due to the presence of a trace of hydrochloric acid, soon became neutral, and continued neutral until a quantity of alkali had been added which was sufficient for the complete solution of the pigment. When the material had become faintly alkaline, small successive portions of a 2 per cent solution of potassium permanganate were added. The first portion was immediately reduced and manganese dioxide was precipitated; but subsequently the reduction ceased and on testing with litmus the liquid was found to be no longer alkaline. Upon adding a few drops of alkali, the reduction of the permanganate continued and manganese dioxide was thrown down. This alternate addition of permanganate and alkali was continued until the solution showed a permanent chameleon color from a slight excess of permanganate, when it was found that the total amount of alkali that was necessary to maintain an alkaline reaction was about twice that required for the solution of the pigment. Immediate reduction of permanganate by the pigment in the cold therefore occurs only in an

alkaline fluid and the alkalinity of the material is diminished during the reaction.

If the results which we shall describe are to be obtained at all, it is important to note that the alkalinity of the material should not be initially too great, for in such a case the permanganate is immediately changed to the green manganate which seems to be entirely inefficient for the oxidation of the pigment. In every experiment therefore that shall be described the pigment was treated in the beginning with twice the quantity of sodium hydroxide that was required to effect its solution. If it should happen that too great a quantity of alkali is used, the error will be shown by the green color of the solution after permanganate has been added, and can be corrected by the addition of a trace of hydrochloric acid. On the other hand, should no precipitate of manganese dioxide occur after eight or ten minutes it is certain that the material is not alkaline and a trace of alkali should be added.

Ten portions of the pigment mixture were treated with the proper amount of alkali and a different amount of two per cent permanganate solution was added to each (see table). In every instance the addition of permanganate reduced the alkalinity of the mixture. At the end of a few minutes the entire solution became gelatinous, due to the formation of a clot of manganese dioxide hydrate; but on agitation the precipitate became flocculent and quickly subsided, so that an examination of the liquid could be made. In every one of the ten experiments the solution became again intensely alkaline and the color was brown from an excess of undecomposed pigment or chameleon from an excess of permanganate. On standing, the oxidation of the pigment continues, so that solutions containing permanganate in excess soon become cloudy, and finally lose the chameleon color altogether.

The results given in the accompanying table show that in a solution of certain definite alkalinity the pigment is acted upon immediately by a relatively large quantity of potassium permanganate and that the acidity of the product is about twice as great as that of the pigment itself; that subsequently a second reaction occurs in which manganese dioxide is thrown out and the alkalinity of the products is thereby markedly increased; and that a slower oxidation follows, more than three times as much permanganate being reduced in the first five minutes as in the succeeding forty-eight hours. We were at first inclined to the opinion that the material with which

these experiments were made was a mixture of at least three different substances, one of which constituted the greatest mass of the pigment and was immediately oxidized, a second was present in much smaller amount and was more slowly oxidized, while a third existed only in traces and was incapable of oxidation by permanganate. The following experiment, however, shows this to be but another instance of the failure of a chemical process to reach completion on account of the accumulation of the products of the reaction. A comparatively large portion of pigment was treated as in Experi-

	Pigment used. c.c.	Permanganate added. c.c.	Color of the solution after clot had formed.	Color of the solution after standing four hours.	Color of the solution after standing 20 hours.	Color of the solution after standing 48 hours.	After acidifying filtered solution with hydrochloric acid.
1	10	4.8	Dark brown				Voluminous precipitate of pigment.
2	10	5.2	Brown				Immediate precipitation of a small quantity of pigment.
3	10	5.6	Chameleon	Brown			
4	10	5.8	Chameleon	Brown			
5	10	6.0	Chameleon	Chameleon	Pale brown		
6	10	6.3	Chameleon	Chameleon	Yellow		Precipitation of a small quantity of pigment on standing.
7	10	6.7	Chameleon	Chameleon	Chameleon	Yellow	
8	10	7.0	Chameleon	Chameleon		Chameleon	
9	10	7.3	Chameleon	Chameleon		Chameleon	
10	10	8.0	Chameleon	Chameleon		Chameleon	

ment 3 of the series given above, and after all the permanganate had been reduced, the manganese dioxide was filtered off and the filtrate acidified with hydrochloric acid. The unoxidized pigment that was precipitated was purified by alternate solution in dilute alkali and precipitation with acid. After finally dissolving in the stated quantity of alkali it was found to reduce permanganate immediately, the reaction exhibiting all the phenomena that have been noted in connection with the original pigment.

It has already been shown that by the complete oxidation of this pigment in alkaline solution with chlorine only such simple sub-

stances as carbon dioxide and ammonia are to be found.[1] This, taken in connection with numerous failures to isolate any organic compound when the oxidation had been conducted as in Experiments 8, 9 and 10, especially where the excess of permanganate was removed after acidifying, led us to Experiment 3 as the one most likely to be successful.

Separation of the Products of Oxidation.

A quantity of pigment emulsion which contained 10.6 grams of dry pigment was treated in ten portions by the method described above in Experiment 3. In each experiment the phenomena already noted were produced, viz., the immediate increase in acidity after the permanganate had been added, the marked increase in alkalinity as the clot formed and the subsequent slow reduction of the excess of permanganate. After standing over night the manganese dioxide was filtered off and the united filtrates markedly acidified with hydrochloric acid. A brown flocculent precipitate of undecomposed pigment was thrown out, leaving a dark red solution. Without filtering, a large excess of barium chloride was added, which formed an additional very dark brown precipitate. The red liquid was then filtered, and after the hydrochloric acid had been nearly neutralized with caustic soda, the material was allowed to stand for several days. The bulky yellow precipitate which had been formed in continually increasing amount as the acidity of the solution was reduced, could now be seen to consist of two distinct kinds of material. The one a yellow bulky flocculent substance which had no tendency to settle when disturbed, and the other a heavy granular substance which adhered to the sides and bottom of the vessel so that a separation of the two substances by decantation could be easily made.

The flocculent portion of the barium precipitate. — The light yellow material was filtered off and extracted successively with one-half per cent, one per cent, and two per cent hydrochloric acid. The part insoluble in two per cent acid was very dark brown; the precipitate obtained by nearly neutralizing the two per cent hydrochloric acid solution was considerably lighter in color, that obtained similarly from the one per cent acid was yellow, while the solution in one-half per cent hydrochloric acid gave a precipitate which was scarcely colored. All of the precipitates were light and flocculent, all contained both nitrogen and barium, and all were thrown down from acid

[1] W. Jones: *Loc. cit.*

solution by alkali before the neutral point was reached. A portion of each acid solution that had been reserved was decolorized as far as possible with animal charcoal and afterwards neutralized with sodium hydroxide. The two per cent acid solution gave no precipitate at all, the one per cent acid solution a small yellow precipitate while the one-half per cent acid solution gave a considerable amount of a straw-yellow barium compound. Thus the original brown pigment, which is precipitated from its alkaline solution by the slightest excess of hydrochloric acid and whose black barium compound is not soluble to an appreciable extent in five per cent hydrochloric acid, is oxidized with the greatest ease by potassium permanganate giving rise to a succession of products. Those formed in the earlier stages of the oxidation are dark in color, difficultly soluble in hydrochloric acid, rich in nitrogen and they yield barium compounds which are also difficultly soluble in hydrochloric acid. On the other hand, the more completely oxidized products formed in the later stages of the oxidation are light in color, easily soluble in hydrochloric acid, poor in nitrogen and yield pale-colored barium compounds that are easily soluble in hydrochloric acid. These experiments furnish a partial explanation at least of the general failure among physiological chemists to obtain concordant analytical results, even when dealing with pigments of the same origin; for a substance which so easily undergoes oxidation might take up oxygen in alkaline solution from the air and the composition of the substance precipitated from such a solution would depend entirely upon the amount of hydrochloric acid that is used for the precipitation.

The granular portion of the barium precipitate. — The granular portion of the barium precipitate was detached from the sides of the vessel, washed with water by decantation until all traces of flocculent material had been removed, and finally dried with alcohol and ether. The substance was gritty, pale yellow in color, and was seen under the microscope to consist of irregular grains. It was finely ground and extracted in the cold with two per cent hydrochloric acid. The acid solution was boiled with animal charcoal until all color had been removed and the acidity was reduced with sodium hydroxide, but the addition of the alkali was discontinued before the neutral point was reached. A perfectly white flocculent precipitate was produced which was so voluminous that scarcely any interstitial liquid could be seen. Upon standing a few moments the bulky precipitate disappeared, and on the bottom of the vessel was seen a small amount of

heavy granular material which, aside from the fact that it was perfectly colorless, scarcely differed in its physical properties from the original granular barium precipitate. A part of the material was dissolved several times in dilute hydrochloric acid reprecipitating each acid solution by reducing the acidity with sodium hydroxide, and in every case where the precipitation was made in a solution that still contained free acid a bulky flocculent precipitate was first formed which upon standing became heavy and granular. The main portion of the granular substance was washed with water until the washings were free from chlorine and subsequently dried with alcohol and ether. The substance is free from nitrogen and chlorine. It cannot contain barium carbonate, because it was precipitated from a solution containing free hydrochloric acid. When heated on the foil it turns gray without melting, sparks, and burns easily to a colorless ash. The ash is completely soluble, with effervescence in dilute hydrochloric acid which shows the substance to be free from barium sulphate and silica. A test on the platinum foil with potassium nitrate and sodium carbonate also showed the complete absence of manganese. This substance which constitutes by far the greatest amount of material, which we found among the oxidation products of the pigment, and which we believe to be a mixture of barium oxalate and barium bioxalate, was analyzed with the following results:

I. 0.2121 gram of substance burned with potassium bichromate gave 0.0816 gram of CO_2 and 0.0175 gram of H_2O.
II. 0.2272 gram of substance burned with potassium bichromate gave 0.0857 gram of CO_2 and 0.0198 gram of H_2O.
III. 0.2659 gram of substance gave 0.2411 gram of $BaSO_4$.
IV. 0.2353 gram of substance gave 2135 gram of $BaSO_4$.
V. 0.2468 gram of substance lost 0.0195 gram on heating.
VI. 0.2824 gram of substance lost 0.0225 gram on heating.

Theoretical for $Ba.C_2O_4.H_2O$		Theoretical for $BaH_2(C_2O_4)_2.2H_2O$	Found.					
			I	II	III	IV	V	VI
C	9.88	13.67	10.49	10.28				
H	0.82	1.71	0.92	0.97				
Ba	56.38	39.03	..	..	53.31	53.35		
H_2O	7.41	10.26	..	..	..	..	7.90	7.97

The following experiment lends a high degree of probability to the conclusion that the substance analyzed is a mixture of the two salts named.

A very dilute solution of ammonium oxalate was treated with barium chloride and the barium oxalate which was precipitated was thoroughly washed and dissolved in boiling two per cent hydrochloric acid. On adding sodium hydroxide in insufficient amount for the complete neutralization of the acid, a light flocculent precipitate was produced which in a few minutes assumed a granular form and settled rapidly leaving a perfectly clear and highly acid liquid. The precipitate was washed until free from chlorine and dried with alcohol and ether. The analysis of three such preparations shows that under these conditions a salt of variable composition is precipitated and it is difficult to imagine how in so simple a case the variation in composition could be due to any cause other than the precipitation of variable amounts of the two oxalates of barium.

I. 0.2846 gram gave 0.2640 gram $BaSO_4$
II. 0.2124 gram gave 0.1774 gram $BaSO_4$
III. 0.1947 gram gave 0.1658 gram $BaSO_4$

	Theoretical for oxalate.	Theoretical for bioxalate.	Found. I	II	III
Ba	56.38	39.03	53.80	49.11	50.07

It was stated that the granular barium precipitate is only partly soluble in cold two per cent hydrochloric acid. The insoluble portion was treated with boiling two per cent hydrochloric acid and the solution decolorized with animal charcoal. On cooling, a perfectly white crystalline material is deposited which consists of a network of beautiful prisms. When the compound is recrystallized from a more concentrated solution in two per cent hydrochloric acid it forms fine needles. After recrystallization the material was washed with water until all chlorine had been removed, then with alcohol and ether. The analysis shows that it is pure barium bioxalate.

I. 0.1847 gram gave 0.0925 gram of CO_2 and 0.0285 gram of H_2O.
II. 0.2133 gram gave 0.1048 gram of CO_2 and 0.0332 gram of H_2O.
III. 0.2107 gram gave 0.1397 gram of $BaSO_4$.
IV. 0.1802 gram gave 0.1196 gram of $BaSO_4$.
V. 0.2111 gram lost 0.0215 gram on heating.
VI. 0.2007 gram lost 0.0204 gram on heating.

Theoretical for $BaH_2(C_2O_4)_2 . 2H_2O$		Found.					
		I	II	III	IV	V	VI
C	13.67	13.66	13.39				
H	1.71	1.71	1.73				
Ba	39.03	..	..	38.99	39.02		
H_2O	10.26	..	..	..	..	10.14	10.11

On the Amount of Oxalic Acid Formed.

Having found that oxalic acid is one of the principal end products of the oxidation of the pigment, it is naturally of interest to know what part of the carbon of the pigment under the most favorable conditions can be converted into oxalic acid. A rough estimate based on the quantity of the two barium salts that have been described led to the conclusion that at least twelve per cent of the carbon of the pigment is to be found after oxidation as oxalic acid. This number is, of course, too low, for no account is taken of great losses in material which are known to have occurred, and the experiments were not made under conditions which might be expected to produce a maximum quantity of the end products. It is at present impossible to make an accurate quantitative estimation; for, as already stated, the pigment does not undergo complete oxidation when the products of oxidation are allowed to accumulate. There is, moreover, a quantity of pigment which is dragged down by the precipitated manganese dioxide and escapes oxidation, for after washing this precipitate many times with boiling water, it yields ammonia in large amount when heated with caustic soda. Nevertheless, it can be shown that at least 20 per cent of the carbon of the pigment is changed to oxalic acid.

An alkaline solution of pigment was prepared of a strength suitable for the oxidation, and an estimation of the carbon of the pigment contained in a measured portion of the solution was made by the method outlined at the beginning of the paper. A number of equal portions of this alkaline solution were treated with various quantities of permanganate and allowed to stand for forty-eight hours. The material for analysis was selected from the vessel which still contained a trace of permanganate. The solution was filtered from the manganese

dioxide. The latter was then extracted with boiling water which showed by its deep brown color that a large quantity of pigment was contained in the manganese precipitate. The precipitate was extracted several times with boiling water, and each extract was highly colored until all the alkali had been dissolved out, when the manganese precipitate still contained pigment since it produced pyrrol when heated alone and ammonia when heated with alkali. This loss of pigment might have been partly avoided by making the oxidation at the boiling point. This method was not employed, however, for the reason that in spite of the common statements that oxalic acid exerts no reducing action on permanganate in alkaline solution, we were able to show that manganese dioxide is copiously precipitated from a boiling solution of potassium permanganate upon the addition of a solution of ammonium oxalate to which a few drops of caustic soda had been added.

The alkaline filtrate, which still contained a trace of potassium permanganate, was treated in the cold with a few drops of a dilute solution of ferrous ammonium sulphate, and after filtering off the small precipitate of manganese dioxide and iron hydroxide, the yellow solution was completely decolorized with animal charcoal. Two equal portions were then measured out for the determination of the oxalic acid. One of these was acidified with acetic acid and treated with an excess of calcium chloride. The precipitate of calcium oxalate was filtered off, washed thoroughly, dissolved in hot dilute sulphuric acid, and titrated with a standard solution of potassium permanganate. The second portion was also acidified with acetic acid and the oxalic acid precipitated with calcium chloride. The calcium oxalate was washed, dissolved in dilute hydrochloric acid, filtered from a trace of insoluble substance (probably silica) and again precipitated by the addition of ammonia. The calcium oxalate was filtered, washed, dried, incinerated, and weighed as calcium oxide. By an obvious calculation it was found in the first experiment that 20.5 per cent and in the second 21.3 per cent of the carbon of the pigment had been oxidized to oxalic acid.

In view of the fact that so many substances yield oxalic acid by oxidation, it is of little consequence that this animal pigment is to be added to the list. But when we consider how few substances yield oxalic acid by oxidation with permanganate in cold alkaline solution, and that at least one-fifth of the carbon of this pigment is so transformed, it would seem that we have in this reaction an unmistakable

expression of internal structure, and we should now be in a position to decide whether the various natural and artificial melanins are in reality similarly constituted, or whether their common color and resistance to reagents in general is to be regarded as accidental.

Theoretical.

It will be remembered that in the course of his most remarkable work on the metabolism of nitrogen, Drechsel [1] was able to show that by oxidation in ammoniacal solution with ammonium permanganate both leucine and glycocoll yield oxamic acid. It is reasonable to assume that had he used a solution of the amido acids in sodium hydroxide and made the oxidation with potassium permanganate he would have obtained oxalic acid. Drechsel also showed how phenol or tyrosine might by alternate oxidation and reduction yield normal caproic [2] acid and that by the passage of an alternating current, normal caproic acid $CH_3(CH_2)_4COOH$, can be made to yield adipic acid $(CH_2)_4 < \begin{matrix} COOH \\ COOH \end{matrix}$, succinic acid $C_2H_4 < \begin{matrix} COOH \\ COOH \end{matrix}$, and oxalic acid $\begin{matrix} COOH \\ | \\ COOH \end{matrix}$. We know also that aspartic acid $\begin{matrix} CH_2 . COOH \\ | \\ CH . NH_2COOH \end{matrix}$ and glutamic acid $\begin{matrix} CH_2 . COOH \\ | \\ CH_2CH(NH_2)COOH \end{matrix}$ are constant products of the hydrolysis of the proteids. It is immaterial whether or not these acids yield oxalic acid by oxidation. Some of them certainly do and it does no violence to any of our conceptions of chemical action to assume that the precursors of all may pass through the intermediate stage and yield oxalic acid as an end product.

It would be difficult, on the other hand, to understand how tyrosine could be changed into adipic acid under the conditions of our experiments, so that if the grouping which in the proteids gives rise to tyrosine is also present in the pigment molecule, some aromatic compound should be found among our oxidation products. In spite of a most careful examination no benzene derivative has been found. The filtrate from the precipitated barium salts was made neutral, decolorized with charcoal and evaporated to dryness. A portion of this residue was treated with concentrated nitric acid which contained

[1] Drechsel: Archiv für Physiologie, 1891, p. 248, with references.

[2] Drechsel: Journal für praktische Chemie, 1888 [2], xxxviii, p. 65.

strong sulphuric acid, and after diluting, the material was made alkaline with ammonia without any change in color. On the other hand, the residue, when heated dry, develops a strong odor of burnt hair, and the fumes yield a marked pyrrol reaction with a pine splinter which has been moistened with hydrochloric acid. A portion of the residue was dissolved in sodium hydroxide and evaporated to dryness. The residue was free from ammonium salts, but contained nitrogen, and a solution of the residue in water holds copper hydroxide in solution with a bluish green color. This work, therefore, taken in connection with the researches cited, indicates with some degree of probability that the pigments are related to the proteids, and that in the pigments the predominant grouping is that which in the proteids gives rise to the aliphatic amido acids.

STUDIES ON THE INFLUENCE OF STRYCHNINE ON THE SPINAL CORD OF RABBITS.

By H. A. HARE.

[*From the Laboratory of Experimental Therapeutics, Jefferson Medical College.*]

THE objects of this research were to determine whether immunity to strychnine may be produced, and to find if possible the exact portions of the spinal cord upon which this drug exercises its chief effect.

The first step was to obtain perfectly healthy rabbits and to determine what might be called the convulsive or poisonous dose.

As the result of a series of experiments it was found that if rabbits weighing from 1500 to 2100 grams, or an average of 1720 grams, received 0.0006 gram of strychnine sulphate hypodermically into the subcutaneous tissues two inches below the spine in the side, convulsions did not occur. This was confirmed by another series of experiments in which rabbits weighing from 1750 to 2300 grams, or an average of 2010 grams, received in a similar manner 0.0008 gram of strychnine without producing convulsions, the dose per gram of body weight being somewhat larger than in the first lot. Although these rabbits of the second series were not convulsed, they were evidently distinctly affected by these doses, as shown by the period of absolute quiet followed by excessive extension of the hind legs when they moved. Evidently an impulse was irradiated from the spinal cord in excess of the needs of the intended movement. The rabbits seemed on the border-line of a spinal explosion.

That the dose of from 0.0008 gram of strychnine to about 2010 grams of body weight is near the convulsive dose is proved by the fact that in a third series of experiments, when rabbits weighing from 1800 to 2200 grams, or an average of 2050 grams received 0.0009 gram of strychnine, in the same manner as before, all were convulsed, and one weighing 1800 grams died. It would seem certain, therefore, that strychnine sulphate given hypodermically into the tissue of the side or back will cause convulsions when given in the dose of about 0.00085 or 0.0009 gram to a rabbit weighing about 2000 grams.

This is rendered still more certain by another series of experiments

in which 0.0006 gram given to rabbits weighing from 900 to 1200 grams, or an average of 1050 grams, caused convulsions followed by death.

The convulsive dose being approximately determined, an attempt was made to produce immunity by repeated doses. Another set of rabbits weighing from 1780 to 2420 grams or an average of 2100 grams now received strychnine during a period of one week; the dose began with 0.00015 gram, and increased as follows:

October 2	each	rabbit	received	0.00015	gram.
3	"	"	"	0.0002	"
4	"	"	"	0.00035	"
5	"	"	"	0.0004	"
6	"	"	"	0.00045	"
8	"	"	"	0.0005	"
9	"	"	"	0.0006	"

These doses did not produce convulsions in any case; but the last dose of 0.0006 gram caused distinct increase of reflex excitability in the smaller rabbits, weighing respectively 1780, 1800 and 2100 grams. The two heavier ones, weighing 2400 and 2420 grams, showed increased respiratory activity. It is noteworthy that the results from the dose of 0.0006 gram given to rabbits weighing about 2100 grams, which had received increasing doses, caused more marked excitement than did 0.0006 gram given to rabbits weighing about 1720 grams which had received but one dose of the drug, or, in other words, the use of frequently repeated increasing doses increased the susceptibility very distinctly.

After two days' interval rabbits which had been receiving increasing doses of 0.0008 gram were given 0.0006 gram. This caused still greater evidence of increased reflex irritability in the larger rabbits, and convulsions in those weighing from 1780 to 2100 grams. Two days later they all received a second dose of 0.0008 gram which caused convulsions in all except one weighing 2420 grams, which, however, seemed just on the border-line of a convulsion. This dose killed the two rabbits weighing 1700 and 1800 grams. It is evident, therefore, that the frequent repetition of the doses increases rather than decreases the susceptibility to the drug, for 0.0008 gram of strychnine to 2100 grams of body weight caused death in all but one of the animals, while the dose of 0.0008 gram to 2010 grams body weight without previous use of the drug did not cause convulsions.

Thinking that the increasing doses might have been too frequently

repeated to permit the acquirement of immunity, I now gave 0.0006 gram every other day for ten days to the rabbits weighing 2150, 2400, and 2420 grams, and to three others weighing 1620,[1] 1750, and 1975 grams, or an average of 2044 grams, which had also received increasing doses, although not included in the experiment previously quoted.

On the first dose of 0.0006 gram those animals weighing 1620, 1750, and 1975 grams were convulsed ; but none died. Each succeeding dose produced similar results, and when the dose was raised on the twelfth day to 0.0007 gram, the rabbits weighing 1620 and 1750 grams died of the convulsions. This still further confirms the conclusions already reached. Whether this increased excitability is due to the cumulative action of the poison is difficult to determine.

The influence of convulsive doses upon these animals was peculiar and worthy of notice. Immediately after the injection of the poisonous dose the animal would begin eating with ordinary zest, but after a few minutes would become very quiet, save that its respirations were somewhat more rapid. In a few minutes more it would assume an attitude of muscular fixation, as if attempting to obtain a greater voluntary control over its muscles. If disturbed, it would hop with greatly increased manifestations of energy in the hind legs, so that it would evidently be propelled farther than it meant to go. After another interval of quiet it would instantly fall into a convulsive seizure which passed off usually in ten or fifteen seconds, and no sooner had the convulsion passed by and the animal got its breath, than it would attempt to get up, very much as a man recovering from an anæsthetic attempts to rise. Power seemed to return at once in the fore limbs, but the hind limbs often remained partially paralyzed for some minutes longer. After power returned to the hind limbs, the animal seemed quite well again, and in the course of twenty minutes was often to be seen eating heartily.

The point of great interest in this connection is that the convulsive effect of the drug should be so fleeting. If the convulsion is the manifestation of the absorption of enough of the drug to produce an explosion of nervous energy by the spinal cord one would suppose that these convulsive seizures would occur again and again, until the system had a chance to eliminate or destroy the poison, or that, hav-

[1] It will be noticed that this rabbit weighed much less than those which died. Its survival perhaps depended upon the fact that it was a gray rabbit, which variety seems to have greater vitality than white rabbits, and greater resistance to strychnine.

ing caused exhaustion of the cord by violent impulses, it would produce paraplegia which would be lasting till the drug was eliminated. Again, it would be supposed if the cord was exhausted temporarily by the convulsion that so soon as it recovered it would again be convulsed; but this was not the case, as already stated. The storm comes and is gone in a few seconds, yet when repeated doses are given, the susceptibility is increased.

The spinal cord of a number of the animals used in the present investigation was examined by Nissl's method by Dr. Simon Flexner, to whom I am under very great obligation for his interest and trouble. Dr. Flexner and the writer were anxious to discover whether any demonstrable change took place in the spinal cells which had been powerfully affected by strychnine. Dr. Flexner found that the changes in the nerve cells, so far as they are demonstrable by Nissl's method of staining, are of slight degree. The majority of cells showed a perfectly normal form and arrangement of stichochrome granules. A small number of cells showed slight central chromotolysis with partial migration of the nucleus toward the periphery of the cell. In no instance was the nucleus in immediate contact with the wall of the cell, and the only cells which showed any change whatever were the cells in the anterior horns. The cells of the intervertebral ganglion were normal.

These experiments are of interest in several respects. In the first place they seem to prove that immunity to strychnine is not produced by increasing doses, secondly, that for this reason physicians who desire to give full doses of this drug may do so at once without fear of producing over-effects, since increasing doses do not, as has been thought, enable the patient to take more than the ordinary individual may take with safety.

Again, the experiments are of interest in respect to certain work recently done by several investigators, notably, v. Czyhlarz and Donath, and Meltzer and Langmann.[1] Von Czyhlarz and Donath concluded as the result of their experiments that the tissues of the body have the power of rendering a poison, notably strychnine, inactive by causing its fixation in the tissues, or perhaps by destroying it locally, provided its rapid absorption and transference elsewhere is not speedily accomplished. Meltzer and Langmann contradict this and claim that if the poison is injected into a part, and then

[1] Meltzer and Langmann: Centralblatt für innere Medicin, 1900, p. 929.

prevented from being absorbed into the general system, the prolongation of life after the reception of a normally lethal dose is produced by interference with ultimate absorption caused by the ligature. It cannot be denied that in part both sets of observers are correct, in so far as the delay in the production of death is concerned; but it would seem probable that the power of the body to oxidize poison in all its capillary networks ought not to be ignored. The studies of Lautenbach many years ago certainly seem to show this. Recently Faust[1] has proved in a series of experiments that the ability to withstand large doses of morphine depends not so much upon blunting of the nervous system or upon becoming accustomed to the drug, as upon an increased ability to eliminate the drug from the body.

I did not find in any of the experiments that the anterior portion of the body was more affected than the posterior portion, except, of course, that the head was markedly retracted. I mention this because a study of a large number of papers published on strychnine during the last fifty years reveals a paper by Girard[2] in which Schiff is quoted as stating that the cramp begins in the anterior extremities, a statement also made by Mayer but contradicted by Frensburg. According to Girard's experiments Schiff's statement is correct. The results of these experiments tabulated by Girard seem to show that the anterior segment of the spinal cord, even when completely separated from the medulla oblongata, reacts to the toxic action of strychnine at a time when the posterior parts appear untouched.

Instead of finding that the strychnine chiefly affected the anterior part of the rabbit's body in the early part of the seizure, I found that it affected the hind legs very promptly, and that the exhaustion paralysis of these parts was marked, whereas the anterior extremities speedily regained their power. This is in accord with Girard's statement that in the frog after the convulsions reflex excitability remains in the anterior parts after it is lost in the posterior parts.

[1] Faust: Archiv für experimentelle Pathologie und Pharmakologie, 1900, lxiv, p. 217.

[2] Girard: Archiv für die gesammte Physiologie, 1886, xxxviii, p. 548.

ON THE RHYTHMIC ACTIVITY OF THE ŒSOPHAGUS AND THE INFLUENCE UPON IT OF VARIOUS MEDIA.

By PERCY G. STILES.

[*From the Physiological Laboratory of the Johns Hopkins University.*]

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Initial Purpose of the Work.

THIS research was undertaken with the intention of testing the influence of various substances in solution upon plain muscle tissue, especially on the musculature of the alimentary canal and its rhythmic properties. It was a matter of particular interest to see whether this form of contractile tissue would show the same dependence upon Na, K, and Ca for its continued activity, as does the heart. It is well established that cardiac muscle makes its most prolonged series of beats when all three of these elements are present in suitable proportions in the surrounding medium. On the other hand, skeletal muscles placed in simple NaCl solution give twitches which have been called rhythmic and which cease on the addition either of $CaCl_2$ or KCl. Hence it was desired to find out whether the plain muscle when immersed in certain baths would act like the heart or like the skeletal muscle, or in a manner peculiar to itself.

The tissue chosen for study was a cross-cut segment from the œsophagus of the frog. Probably any part of the alimentary canal of this animal will exhibit spontaneous movements. Schultz[1] has

[1] Schultz: Archiv für Physiologie, 1897, pp. 1, 307, 322.

studied those of the stomach and Woodworth [1] has employed similar preparations. Botazzi and Grünbaum [2] have used the entire œsophagus of the toad, recording its longitudinal contractions. In the present instance it was soon found that the œsophagus is preferable to any other portion of the canal for the purpose sought. Preparations of the stomach and intestine trace records which are commonly so uneven and interrupted by such long pauses that one cannot interpret them with any confidence. In contrast to these, the circular fibres of the œsophagus have a remarkable rhythmic property and often give for hours together tracings that are as uniform as heart-records. Changes of the solution register their effects clearly and promptly in such tracings. The contractions made by the œsophagus are much more rapid as well as more regular than those of lower segments of the alimentary canal and it will be shown that in the œsophagus itself the rate increases toward the pharynx. Botazzi [3] has noted that in the œsophagus of a gasteropod (Aplysia) a stronger tone characterizes the oral end and he hints that it may have a more rapid spontaneous rhythm, but this he could not demonstrate. It is very obvious in the frog.

Method of Experiment.

As a rule, two rings were cut from the upper part of the œsophagus and opened to form strips which were made to trace simultaneous records. The method of registering was similar to that employed by Howell [4] and Greene [5] for strips from the terrapin heart and still more closely resembled that described by Lingle.[6] A thread was tied around each end of the strip and it was made fast below to the tip of a glass rod bent so as to dip into a small conical measuring-glass containing the bath. The free end was attached to a light lever of split straw modified from the type used by Greene. The lever was borne by the arm of a Basel stand and the writing-point of tin foil traced on the lightly smoked surface of a drum that made one revolution in twelve hours.

[1] Woodworth: This journal, 1900, iii, p. 26.

[2] Botazzi: Journal of physiology, 1897, xxi, p. 481; Botazzi and Grünbaum: Journal of physiology, 1899, xxiv, p. 51.

[3] Botazzi: *Loc. cit.*

[4] Howell: This journal, 1899, ii, p. 47.

[5] Greene: This journal, 1899, ii, p. 82.

[6] Lingle: This journal, 1900, iv, p. 265.

The ordinary precautions were observed in making solutions; the water was distilled from glass vessels and the salts were recrystallized when possible. A point should be noted here in regard to the use of $CaCl_2$. The fused salt is more easily used because it is anhydrous and can be weighed accurately. It has been found, however, in this laboratory, that solutions made with fused $CaCl_2$ often show an injurious property which is very likely due to their containing a trace of free chlorine. It has therefore been customary to make up a stock solution of crystallized $CaCl_2$, determine gravimetrically its content of CaO, and dilute to the percentage strength desired. This method was followed in the present work.

The Character of the Contractions.

Rate. — The contractions made by strips from the part of the œsophagus are much more rapid in all their phases than those which Schultz describes as typical of the stomach and Botazzi as characteristic of the longitudinal fibres of the œsophagus. These authors give from one to two minutes as an average period of contraction at the room temperature while the circular preparations used in my experiments may make six complete contractions in a minute. It is unusual to have both strips beat as fast as this. If the upper one reaches a rate of six per minute, the companion strip taken immediately below is likely to contract only four or five times in the same interval. (In one case in which the ratio was determined the upper segment contracted 78 times while the adjacent segment contracted 57 times. Another ratio observed was 100 : 66). There is a much greater difference between the pharyngeal end and the region adjoining the stomach, one centimetre distant in a small frog. Segments thus chosen gave the following results.

	Contractions.	
	Upper.	Lower (8 mm. distant).
1st hour	244	94
2d hour	236	111
3d hour	202	99
4th hour	199	87

Comparison of these two records shows that the contractions of the lower segment were the more extensive, and they probably represent as much work as the first series.

One is in doubt whether to homologize contractions which may occur as often as six times a minute with the more rapid beats of heart muscle or its slower variations of tone. Secondary tone-waves are frequently observed in the œsophagus and at such times the tracing is very suggestive of that made by the auricle or sinus muscle of the terrapin; but the contractions are longer than those of the auricular strip as the primary rhythm is one fourth to one half that of the venous end of the heart. Botazzi classed the contractions of his longitudinal preparations of the toad's œsophagus as tone-waves and the more lively movements of the gasteropod œsophagus as primary beats. The contractions of circular preparations from the frog are intermediate between the two with respect to rate and it is perhaps not desirable to insist upon classifying them.

Amplitude and intensity. — The preparations used have been somewhat small, averaging 10 mm. in length by 2 mm. in thickness. They have been found to work best against a tension of 200–500 mg. Tension is certainly an important factor in developing contractions, though Woodworth has shown that stomach preparations continue to work when relieved of load by after-weighting. The extent of the shortening is very variable; it often exceeds 10 per cent of the original length in vigorous samples. The tracings as obtained represented this movement multiplied 15–20 times.

Small frogs have constantly given better results than larger ones and a seasonal variation is marked. Schultz found that in the spring the plain muscle of the frog is quite unsuitable for experimental uses. This has been emphatically confirmed in the present observations. After the first of February the period of spontaneous contraction became lessened, and with warm weather in April and May no significant work could be done.

Behavior in Solutions of NaCl and the Effects of Adding $CaCl_2$ and KCl.

NaCl. — A 0.7 per cent solution was used. Small variations of this concentration were without evident effect. Placed in such a bath the preparation sometimes relaxed without beating, sometimes gave a series of contractions which grew weaker and were always accom-

panied by a progressive fall of tone. These contractions were usually irregular. They seldom registered beyond the first hour, but minute movements might be noted during three or four hours. If the solution was then made faintly alkaline — Na_2CO_3 to the extent of 0.002–0.01 per cent — the feeble and uneven contractions usually gained some strength and there was sometimes a partial recovery of tone. But the stimulating effect of alkalinity is transient. Here the œsophagus agrees well with the heart, for many workers, notably Gaule[1] and Martius,[2] have shown that the heart-beat continues longer in an alkaline than in a neutral medium.

CaCl. — If $CaCl_2$ in the proportion of 0.015–0.03 per cent be added to the NaCl solution in which a strip has come to rest, rise of tone and irregular movements may be induced, but not a definite rhythmic series.

KCl. — Potassium salts, as in the case of the heart, tend to abolish tone and hasten the cessation of the rhythmic contractions.

Behavior in Ringer's Mixtures.

It is only in the presence of both Ca and K that the contractions continue regular and forcible for many hours. The standard Ringer's[3] solution used in the laboratory is a favorable medium. The NaCl in this mixture is 0.7 per cent, the $CaCl_2$ 0.026 per cent and the KCl 0.03 per cent. When a strip, fresh from the animal's body, is suspended in this solution it commonly shows a gradual rise of tone, reaching a maximum within twenty minutes. Near this maximum the rhythmic contractions begin to be apparent. They may be very small at first and increase for an hour or more before reaching their full force. The gain in amplitude is often seen in both phases — successive contractions are higher than those preceding and successive relaxations are more complete. When the rhythmic beat is well established, the tone and also the height of the contractions may remain practically unchanged for a long time. The rate, however, is always lowered before the next day. Sometimes, in the later portions of a record, the contractions show a tendency to fall into groups with intervening pauses. This is most noticeable toward spring, when the frogs are not in the best condition.

[1] Gaule: Archiv für Physiologie, 1878, p. 291.

[2] Martius: Archiv für Physiologie, 1882, p. 543.

[3] Ringer: especially Journal of physiology, iv, pp. 29, 222; v, p. 247; viii, p. 15; xviii, p. 425.

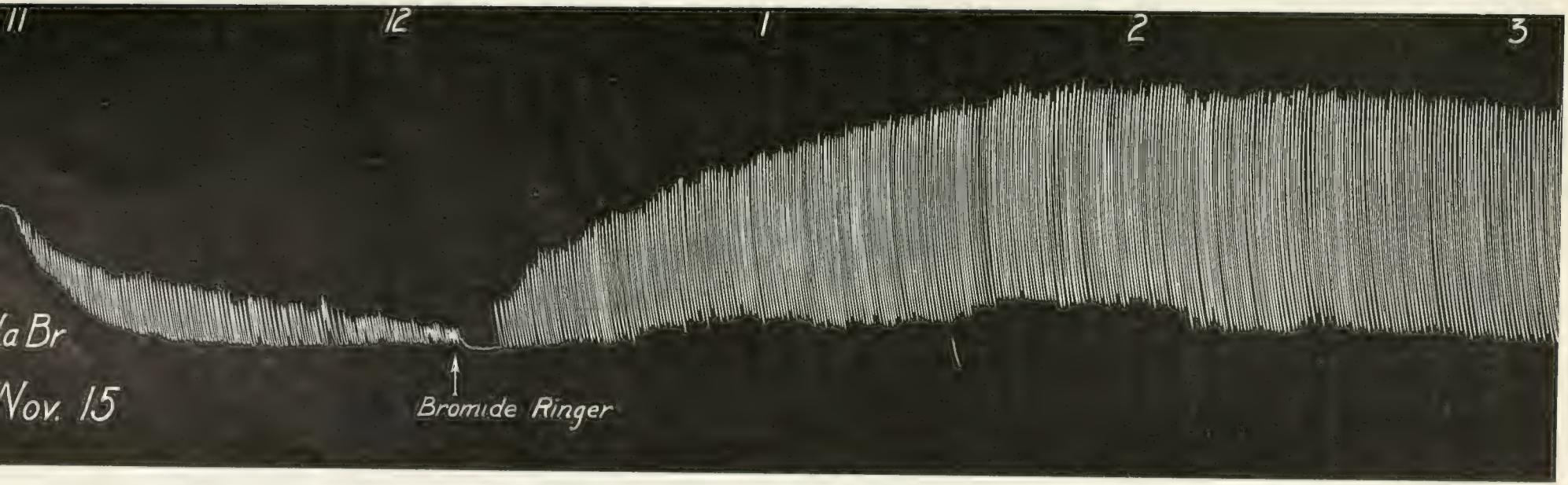


FIGURE 1. — Showing weakening in the absence of Ca and K, and recovery in Ringer's mixture (bromide Ringer). Figures above indicate hours of the day.

Duration of Activity. — Woodworth found that his preparations from the stomach contracted automatically in a moist chamber for from eighteen to thirty hours. This is about as long as the transverse œsophageal preparations can be relied upon to contract well in an unchanged bath of Ringer's solution. When a strip has come to rest it may often be made to contract again by merely substituting for the original mixture a fresh mixture of the same concentration. Perhaps this may be because of the dissolved oxygen thus brought to the tissue. A revival secured in this way is not likely to last long. A better one follows an increase of Ca in the bath. By successive additions the amount of $CaCl_2$ in solution may be brought up to 0.1 per cent and the tissue roused by each addition to renewed activity for an hour or two. In concentrations exceeding 0.1 per cent $CaCl_2$, the element seems to lose its property of heightening tone and its influence becomes depressing, if not toxic. When a strip has ceased to be active in a bath containing Ca in excess it is not notably excited when transferred to one containing less of that element or to simple NaCl solution. By increasing the Ca, as described, a strip may be kept in activity for from thirty-six to forty-four hours. In rare cases the last named period was exceeded; one strip made movements until the forty-ninth hour, another to the fifty-first. At the best these preparations do not equal the terrapin heart strips in the duration of their activity.

Effect of altering the ratio $\frac{KCl}{CaCl_2}$. — The specific effect of K is best studied by using different amounts of its chloride in the presence of a constant quantity of $CaCl_2$. As the KCl is increased above the usual 0.03 per cent the tone is lowered and the contractions reduced until they cease. Complete inhibition is usually reached when the KCl is present in twice the amount of the $CaCl_2$. Thus with 0.03 per cent $CaCl_2$ the tissue becomes relaxed when the KCl exceeds 0.06 per cent. With 0.05 per cent $CaCl_2$ as much as 0.1 per cent KCl may be required to inhibit. When a strip which is still contracting in NaCl solution is transferred to Ringer it usually pauses for a few minutes before beginning the more vigorous series of beats. This inhibition is well accounted for by supposing that the K-salt diffuses into the tissue more rapidly than the Ca-salt, and so comes to be in temporary excess.

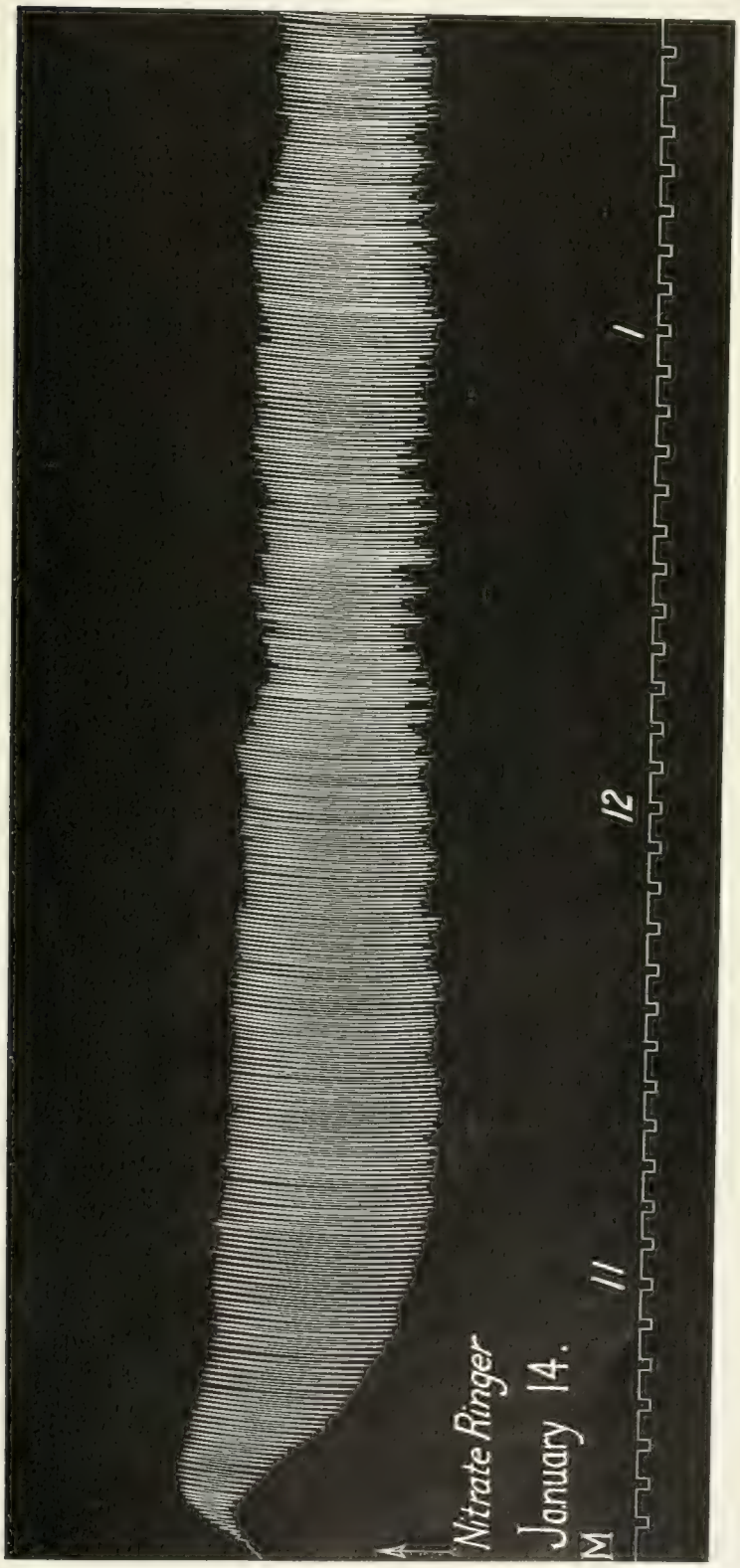


FIGURE 2. — Typical beginning of record in Ringer's mixture. Time-record indicates intervals of five minutes.

Attempts to find a Substitute for NaCl in the Medium.

A series of experiments was made in which it was aimed to substitute for NaCl other compounds in isotonic solution. If NaCl is an indifferent body and useful only in keeping up the osmotic pressure of the medium one may reasonably hope to find one or more compounds among the non-electrolytes capable of performing this simple function and so of taking its place. In the present instance, as in the researches of Loeb[1] and Lingle,[2] no such substitute was found. This negative result favors the inference that Na is necessary in some way to the maintenance of rhythmic activity. Yet such a conclusion may always be challenged while the number of compounds studied remains small and while there is the possibility that each one possesses an inhibitory property.

Sugars. — Most of these experiments were made with dextrose, to which $CaCl_2$ and KCl were added in various proportions. When a strip which has been active in Ringer is transferred to a dextrose solution (3.7 per cent) its tone is usually increased for a time and the contractions become slowed and greatly augmented in amplitude. They may continue for more than an hour; but presently they cease, the tone falls, and the preparation rests. If NaCl is substituted there may be a few movements, but not a noteworthy revival. Nevertheless the muscle has not been injured by the dextrose, for it will resume a powerful beat if placed in Ringer and one is often impressed with the idea that the sugar has been distinctly beneficial.

When it was found that a normal rhythmic beat could not be maintained in dextrose solutions (+$CaCl_2$ and KCl), mixtures of dextrose solution and normal salt to which $CaCl_2$ and KCl had been added were tried. It was soon plain that dextrose interfered with the contractions even when as little as one fourth of the NaCl had been replaced by it. This fact renders it probable that dextrose has a direct inhibitory action. Solutions of cane-sugar and of galactose appeared to be quite like dextrose in their action. Strips immersed in them always come to rest in spite of changes in the amount of $CaCl_2$ and KCl employed.

[1] Loeb: Fick's Festschrift, Braunschweig, 1899; Archiv für die gesammte Physiologie, 1897, lxix, pp. 1, 357; 1899, lxxv, p. 303; 1900, lxxx, p. 229; This journal, 1900, iii, pp. 327, 383.

[2] Lingle: *Loc. cit.*

Other non-electrolytes. — A solution of glycerin isotonic with Ringer and containing $CaCl_2$ and KCl was found to inhibit the contractions immediately. The after-effect was not marked. The same is true of ethyl and methyl alcohols. Urea inhibited the beats and depressed the tone of the preparations.

LiCl. — Less interest centred in the electrolytes, and of these only one, LiCl, was tried in place of NaCl. The strips were found to be very tolerant of LiCl, but they always came to rest when the NaCl of a Ringer's solution had been wholly replaced by this salt. They will beat, however, in Ringer mixtures containing as much as two parts of LiCl to one of NaCl, particularly if the alkalinity is as much as 0.01 per cent Na_2CO_3.

Within the range of these experiments, therefore, *the œsophagus has never been made to beat, except for short periods, in media containing no Na.*

Attempts to Prolong Activity.

Why the strip finally comes to rest in the most favorable medium is a question of some interest. Is its fuel substance exhausted, or is its activity hindered by accumulated waste-products? The good effect, previously described, of renewing the solution may be due to a removal of waste-products of metabolism, but it is not probable that the katabolic products of 40 c.mm. of muscle should greatly affect 15,000 c.mm. of solution, unless in its reaction. It is more natural to suppose that a fresh solution promotes activity by supplying oxygen to the tissue. So it seemed likely that the rhythmic activity might be promoted and prolonged by insuring an abundant supply of oxygen to the preparation. To test this suggestion a Ringer's solution was diluted, then boiled down to its former concentration and covered with a layer of oil. In this gas-free bath the tissue would not work. In a second series of experiments H_2O_2 was added to a Ringer's mixture in small amounts. A neutral solution of H_2O_2 was prepared by a method recommended by Bredig and von Bernick. The amount present was determined by titration with potassium permanganate.

It was found that the presence of a small amount of H_2O_2 inhibits the strips whether the addition is made early or late in the experiment. No strip ever contracted in the presence of 0.03 per cent H_2O_2 and 0.01 per cent is markedly depressing. The oxygen set free by a 0.03 per cent solution amounts to about ten volumes per cent. The H_2O_2 is evidently broken up by the tissue, for the strip becomes

encrusted with bubbles of the gas. This observation is in accord with the fact that excess of oxygen inhibits intestinal peristalsis.

Other experiments were made to determine whether the period of activity could be extended by employing Ringer's mixtures in which colloidal platinum was suspended. It was thought possible that this material, which has so many of the properties of enzymes, might act as an oxidase and that its presence might favor a long-continued and vigorous activity of the œsophageal muscle. These experiments yielded negative results. The contractions seemed to go on as well in the absence of colloidal platinum as in its presence, and for an equal length of time. A slight addition of $KMnO_4$ (0.002 per cent) to the bath seems equally without effect, though the permanganate is steadily decomposed. It is natural to conclude that the strip finds a sufficient supply of oxygen in the ordinary bath, provided the solution be occasionally renewed and that its final coming to rest is not due to asphyxia.

Substitution of other Na-salts for NaCl.

An extended series of experiments was undertaken to find out whether the anion, Cl, in NaCl is necessary to the maintenance of rhythmic activity or whether other Na-salts may be used in place of the chloride. The method employed was to make up solutions containing $CaCl_2$ and KCl as in the ordinary Ringer's mixture, but substituting for NaCl the Na-salt to be tested. Care was taken to have these solutions isotonic, and whenever there was uncertainty in this matter recourse was had to freezing-point determinations. It must be noted that the Cl ions introduced with the K and Ca were constantly present. These represent about one fifteenth of the total Cl ions in the standard Ringer's mixture used.[1] These substituted solutions will be spoken of for convenience as nitrate Ringer, bromide Ringer, etc., according to the salt used in place of NaCl.

$NaNO_3$. — It was found that certain salts of sodium could be substituted for NaCl with little apparent influence on the height, rate, and duration of the rhythmic contractions. One of these is $NaNO_3$. A solution isotonic with 0.7 per cent NaCl makes an excellent basis for

[1] That the presence of Cl ions in the surrounding medium is not essential to the rhythmic contractions was demonstrated in a subsequent experiment in which a Ringer's mixture was used, composed of the nitrates of sodium, calcium and potassium. In this solution the œsophageal strip gave an excellent series of contractions lasting over twenty-four hours.

a Ringer's mixture, the $CaCl_2$ being 0.026 per cent as usual and the KCl 0.03 per cent. Careful comparisons of records traced by strips in normal (chloride) Ringer with those made in nitrate Ringer fail to show any differences that can be called characteristic. On the whole it seems that the nitrate may have a slightly stimulating influence, but this is not clearly established. If a strip is first exhausted in NaCl and then transferred to $NaNO_3$ it is not roused to rhythmic contractions though its tone may be heightened. Ca and K must be supplied to establish a vigorous beat. Nitrate Ringer was used in many experiments and it is certainly not less satisfactory than the common solution. Apparently the tissue can part with many of the Cl ions originally present in it and receive a corresponding number of NO_3 ions without losing its power to execute a long series of rhythmic contractions.

NaBr. — Another salt that can well replace NaCl in a Ringer's mixture is NaBr. The solution used was equimolecular and approximately isotonic with 0.7 per cent NaCl. It has been stated, especially by Loeb,[1] who studied its action on skeletal muscles that the Br ion excites the tissue more than the Cl ion and lowers its irritability in a shorter time. In the present case too, it seemed that the œsophageal muscle might be stimulated to a heightened tone and a more vigorous beat by bromide Ringer; but it did not appear to be exhausted in less than the average time. No distinction between the action of $NaNO_3$ and that of NaBr can be pointed out.

NaI. — It is possible to replace the NaCl of Ringer's solution by NaI. This result was somewhat unexpected, Loeb having described the iodide as more toxic than the chloride or bromide, while Walden found its action on the heart unfavorable. The plain muscle, nevertheless, maintains its contractions for hours in an iodide Ringer. Comparison of the movements made in the iodide with those in bromide Ringer gave the impression that the iodide has a tendency to increase tone and to lengthen the period of maximal shortening so that the tracing of each contraction shows a slight systolic plateau. This peculiarity was not constant.

$NaClO_3$. — Finally NaCl may be replaced by $NaClO_3$. It was formerly supposed that chlorates might be decomposed by living tissue and contribute oxygen to its metabolism. This opinion does not seem to

[1] Loeb: Fick's Festschrift, Braunschweig, 1899; Archiv für die gesammte Physiologie, 1897, lxix, pp. 1, 357; 1899, lxxv, p. 303; 1900, lxxx, p. 229; This journal, 1900, iii, pp. 327, 383.

be held by recent writers on pharmacology and it receives no support from the behavior of the œsophageal muscle in the presence of $NaClO_3$. The strips can be transferred from chloride to chlorate Ringer and back again without significantly changing the character of the record ; it is not apparent that one is more favorable than the other. It may be remarked that the record in chlorate Ringer is likely to be irregular, large contractions mingling with small. One of the longest records secured was that of a preparation in chlorate Ringer, the strip being active until the forty-ninth hour.

Salts capable of replacing much of the chloride but not all. — The four salts already mentioned ($NaNO_3$, NaBr, NaI, $NaClO_3$) were the only ones found which could be freely used in place of NaCl. But a number were found capable of replacing a large fraction of the chloride. These were organic salts including three members of the fatty series — formate, acetate and butyrate — and the tartrate and lactate. A description of the effects of sodium acetate will answer for any of the others. A solution of about 1.2 per cent is isotonic with 0.7 per cent NaCl. If a Ringer's solution is made up with acetate instead of NaCl, retaining $CaCl_2$ and KCl in the usual amounts, the active strip soon comes to rest when immersed in it. There may be a transient exaggeration of the movements, but they are presently slowed and the tone declines, leading to a standstill within twenty minutes. When such a relaxed strip is returned to normal Ringer it does not at once revive. It may be quiescent for an hour, but the beat is almost sure to be renewed and there seems to be no lasting effect from the acetate. The tissue will tolerate a large percentage of sodium acetate if a certain amount of the chloride (or interchangeable salt) is retained in the medium. The proportion of the NaCl that may be replaced by organic salts without checking the contractions is variable It is safe to say that one half the NaCl may be so replaced by acetate without markedly depressing the activity of the strip. Sometimes the substitution can be pushed farther and strips may beat for hours in solutions containing two or even three parts of the organic salt to one of the inorganic. This was shown most successfully in trials with sodium formate and sodium butyrate. In the presence of an excess of the organic salt, the rate at which the contractions occur is usually slowed. It should be noted that these sodium salts of organic acids are largely dissociated in the concentration employed. This precludes the inference that the inhibition brought about by them is due to a diminution in the number of Nations.

Certain salts were found to be distinctly inhibitory when present in small amounts. Some of these are precipitants of Ca, and their action may be attributed to the removal of this element. Others do not form insoluble Ca compounds and their effect must have some other explanation. Consequently the salts unfavorable to rhythmic activity may be classed in two groups.

Precipitants of Ca. — Among the salts used in place of NaCl was Na_2SO_4. $CaSO_4$ is sparingly soluble in water (1 : 400). It is probably less soluble in a solution of Na_2SO_4. At any rate, the effects of sulphate Ringer are most readily explained on the supposition that it is a partial precipitant of Ca. When a change is made from a chloride or nitrate Ringer to one with a sulphate basis and the usual amounts of $CaCl_2$ and KCl, the active preparation soon comes to rest with loss of tone. If more $CaCl_2$ is added until the amount is 0.1–0.15 per cent (from four to six times the quantity in ordinary Ringer) the strip recovers and the record runs a typical course. It is true that in this case the Cl ions are increased, but the recovery is perhaps due to the added Ca. Very recently Miss Moore [1] has found that a small quantity of a soluble sulphate added to NaCl solution is favorable to the continued activity of the lymph-hearts of the frog and indeed may take the place of Ca. No such fact was observed in the present investigation. On the contrary, the results are in harmony with the old observation that blood neutralized with H_2SO_4 is depressing to the heart in a greater degree than blood neutralized with other acids.

The original "Ringer's mixture" was saturated with $Ca_3(PO_4)_2$ and held a sufficient quantity of this salt in solution. It is probable that this is not so soluble in Na_3PO_4 solution as in NaCl, for it is visibly precipitated from the bath when Na_3PO_4 is added. A small addition of Na_3PO_4 inhibits the movements of the strips. As little as 0.01 per cent reduces them. Na_3PO_4 is strongly alkaline and when it was to be added freely it was neutralized with H_3PO_4. The effect of such a neutral phosphate Ringer is instant inhibition. Recovery in normal Ringer is prompt and good. Phosphate solutions must be classed with solutions of Na_2SO_4 as partial precipitants of Ca.

The oxalates are the best precipitants, and the inhibitory effect of this radicle is as definite in the case of the œsophagus as Howell [2]

[1] Miss Moore: This journal, 1901, v, p. 196.

[2] Howell: Journal of physiology, 1893, xiv, p. 219, *note*.

has shown it to be for the heart. The addition of 0.01 per cent $Na_2C_2O_4$ to the medium speedily brings the most vigorous preparation to rest with lowered tone. There is no evidence that the oxalate has any harmful after-effect for a fresh Ringer's solution quickly rouses the resting strip and the record immediately assumes the character it had before the interruption. It should be mentioned at this point that $Na_2C_2O_4$ does not inhibit the movements of a skeletal muscle when it is twitching in NaCl solution, but actually increases them. This fact emphasizes the fundamental difference between the contractions of skeletal muscles which occur for long periods in a bath of NaCl alone (*i. e.*, a condition which is not physiological) and those of the œsophagus which, so far as observed, are never of long duration, unless Ca and K are supplied in physiological amounts.

Salts that inhibit without precipitating Ca. — Several salts show detrimental effects not to be explained as due to the removal of Ca.

$NaNO_2$ is one of these. It has the recognized pharmacological action of the nitrites, causing profound loss of tone, slowing of contractions and, presently, standstill. Inhibition is promptly brought about by as little as 0.05 per cent of the $NaNO_2$. Recovery is possible.

The bile-salts, sodium glycocholate and sodium taurocholate have an effect strikingly similar to that of the nitrite. Minute quantities in the solution (0.05–0.1 per cent) inhibit with lowering of tone, and when the strip is transferred to normal Ringer the recovery is gradual and imperfect. These doses are exceedingly small from the standpoint of the number of molecules which they contain, for a solution isotonic with 0.7 per cent NaCl approaches 10 per cent. Hence it is impossible to replace with a molecule of bile-salt one molecule of NaCl in a hundred.

Sodium benzoate is another salt which depresses and finally inhibits the preparation exposed to its action. When a portion of the NaCl ($\frac{1}{10}$–$\frac{1}{4}$) in a Ringer's mixture is replaced by sodium benzoate the contractions become exaggerated and slowed until they have the character of large irregular tone-waves. The strip comes to rest sooner or later according to the proportion of the benzoate introduced. The related salt, sodium salicylate, is much more toxic than the benzoate and the introduction of 0.05 per cent of it suffices to cause abrupt inhibition.

Borax. The slowing and deepening of the contractions observed when sodium benzoate is present is seen in a more remarkable

manner when the solution contains some borax. This sodium salt, the tetraborate ($Na_2B_4O_7$), is strongly alkaline, and, as in working with Na_3PO_4, it is necessary to neutralize its solutions in order to observe the effect of the anion apart from that of the reaction. HCl was added to secure a neutral mixture which therefore contained several ions but all indifferent or favorable to the normal process excepting the tetraboric acid ion. The tracings show how the presence of this ion transforms relatively quick movements of the tissue into enormously prolonged tone-waves each occupying several minutes. The slowing affects chiefly the phase of relaxation and reminds one of the effect of veratria on skeletal muscle.

Are these Contractions of Neurogenic Origin?

These experiments have little bearing on the much-discussed question as to the source of the stimulus causing these spontaneous movements. Engelmann's researches [1] on the mammalian ureter, Sertoli's [2] on the retractor penis of the horse, and those of Botazzi and Grünbaum on marine invertebrates and on the œsophagus of the toad have tended to show that plain muscle has a rhythmic property apart from the influence of ganglion-cells. Schultz has taken the opposite ground, claiming that the frog's stomach makes no movements unless stimulated by its intrinsic nerve-cells or artificially from without. He believes that he has demonstrated this fact by poisoning strips of stomach with atropine and nicotine. Under such conditions he found that spontaneous movements cease, while the tissue remains irritable. He regards atropine and nicotine as specific poisons for nerve-endings and nerve-cells respectively. Botazzi has objected to the inferences of Schultz and has pointed out that the movements of the alimentary canal are not inhibited unless extremely large doses of atropine and nicotine are applied and that the effect of these poisons is transient, passing off as soon as the strip is washed in salt solution. These objections of Botazzi seem to be well grounded. It was found in some supplementary experiments that a frog cannot be so profoundly poisoned with atropine, given hypodermically, that its œsophagus will not give a normal series of spontaneous beats. Large injections of nicotine, which paralyze the skeletal muscles and make the heart resistant to all attempts to

[1] Engelmann: Archiv für die gesammte Physiologie, 1869, ii, p. 243.

[2] Sertoli: Archives italiennes de biologie, 1883, iii, p. 78.

inhibit it, do not modify the records obtained from œsophageal strips. It is only when these poisons in considerable concentration are brought into contact with the tissue in a bath of Ringer's solution that the movements are inhibited. Even then, as Botazzi stated, recovery is promptly secured when a fresh Ringer's mixture is substituted for that containing the alkaloid. The question under discussion may well be left open. But it appears that Schultz's practice of applying various solutions by means of a brush to a strip of muscle in a moist chamber is open to criticism, because no regard is paid to the osmotic relations of the tissue and the fluid, and because the poisons are brought to the cells in an excessive concentration. An isotonic bath has obvious advantages over the " painting on " method, especially in the fact that it has sufficient bulk to prevent appreciable changes of its composition as a result of exchanges between it and the tissue. The thin film of solution laid on with a brush, must be readily subject to such changes.

Incidental Observations.

Effect of temperature. — A single experiment in which the temperature was varied showed that the circular coat of the œsophagus reacts to temperature changes like other smooth muscle preparations, losing tone when warmed and contracting more rapidly up to a certain point, in this case 26° C. The curve reproduced to show the relation of rate to temperature approximates to a straight line indicating a constant increment of rate for each degree's rise. This property is observed in cardiac muscle. Schultz has studied the effect of temperature on the contraction period of his preparations, and the chief interest in comparing his results with this isolated experiment lies in the fact already mentioned that the rhythm of the œsophagus is much more rapid than that of the stomach.

Effect of concentration and dilution. — It has been stated that some variation of the percentage strength of the solution may be tolerated. A solution containing 0.9 per cent NaCl is readily substituted for the usual 0.7 per cent. The effects of more decided changes were also noted. In one experiment nitrate Ringer isotonic with 0.7 per cent NaCl was alternated with one isotonic with 1.05 per cent NaCl and another isotonic with 0.35 per cent NaCl. The three solutions contained $CaCl_2$ and KCl in equal amounts. The concentrated Ringer caused a spasm such as the heart exhibits when irrigated with a strongly alkaline solution or with one containing Ca, but no K. The

dilute Ringer caused relaxation and a weakened and irregular beat. These results were somewhat unexpected, for distilled water throws contractile tissues into the state of tone which Ringer termed "water-rigor."

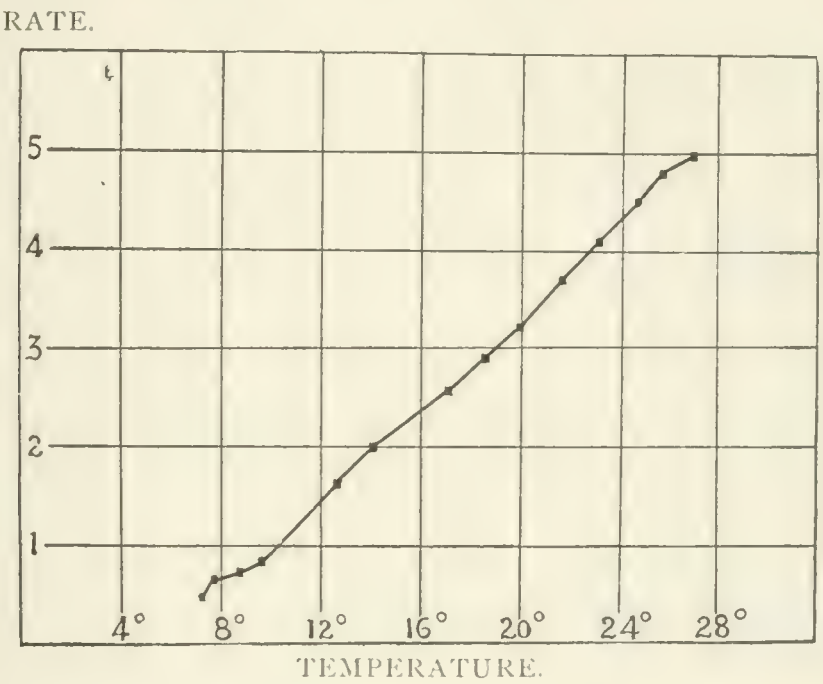


FIGURE 3. — Curve showing the relation between temperature and the rate per minute of the spontaneous contractions. The bath was nitrate Ringer. (Abscissas indicate temperature; ordinates, rate per minute.)

Analogies beween the œsophagus and the heart. — The correspondence between the œsophagus and heart which suggested itself to Botazzi has been emphasized in the course of the present work. The venous end of the heart and the oral end of the œsophagus are both characterized by their more rapid spontaneous rhythm and their tendency to exhibit changes of tone. There is also some indication that the upper part of the œsophagus determines the rhythm of the lower part while there is continuity between the two. The following experiment bears out this statement. An œsophagus was opened by a longitudinal cut and the contractions of its circular fibres recorded at two points between which the preparation was cut part-way through. Later the separation was made complete. After this last step the band from the lower level made fewer contractions than its fellow, though previously it had approached the same rhythm. An experiment with longitudinal strips like those used by Botazzi points in the same direction. Two preparations were made and so placed that the upper half of one and the lower half of the other could

be readily cut away. After two hours and a half this was done. In one case the original oral end remained attached to the lever and in the other the lower portion. The oral end continued to contract, the lower part rested. Such experiments are highly suggestive of the behavior of the heart on the application of the Stannius ligature.

An effort was made to see whether the same resemblance to the heart could be demonstrated in the effect of different solutions on rings from various levels. Howell has found that the sinus and auricle of the terrapin heart beat well in Ringer's mixture from the outset of an experiment while the ventricle usually remains quiescent in Ringer unless it has been already exhausted in saline. But all parts of the œsophagus, like the venous end of the heart, beat at once in Ringer. It is probable that the lower part contracts for a longer time in NaCl solution, but the correspondence between this region and the ventricle is incomplete at best. It was desired to find out which of these segments is more sensitive to Ca, but the results obtained are not conclusive. As little as 0.008 per cent $CaCl_2$ may revive a strip which has come to rest in NaCl, but, as has been stated, the standard mixture containing 0.026 per cent $CaCl_2$ is more effective.

Conclusions.

1. The œsophagus of the frog has a rhythmic property which is more marked than is the case with the remainder of the alimentary canal. In the œsophagus itself this property is most marked at the pharyngeal end which has a more rapid spontaneous rhythm than the parts below. The rhythm of this part (4–6 per minute) is such that it is hard to decide whether the contractions should be denominated as beats or as oscillations of tone.

2. The presence of Ca and K is necessary to the maintenance of rhythmic contractions for any length of time. As in the case of the heart, $CaCl_2$ by itself tends to heighten tone and fuse contractions, while KCl alone tends to abolish tone and inhibit movements. If either of these salts is concentrated above 0.1 per cent its action becomes uncertain and may be considered toxic.

3. No substitute for Na has been found. It is probable that this element, in addition to its undoubted osmotic importance, is essential to the active tissue in a specific way. The evidence at present does not justify a definitive choice between the two theories of rhythmic activity which have been advanced. It may be that Na is a prime

factor and that Ca and K are needed only to neutralize its harmful properties (Loeb's view). But it is more natural to interpret the results as pointing to a stimulating rôle for Ca and an inhibitory action for K. The depressing effect of NaCl alone is so immediate, and failure to beat at all in it is so frequent, that one is inclined to attribute a direct action to the Ca rather than one which is indirect. The agreement between the œsophagus and the venous end of the heart is so complete that one is led to the same conclusions that Howell reached as the result of his study of the sinus and auricle.

4. The Cl ion is not specifically required for normal rhythmic activity. In place of NaCl we may use the nitrate, the bromide, the iodide, or the chlorate.

5. Certain organic salts of Na may replace from one half to two thirds of the chloride without checking the contractions, though they usually slow them. If the substitution be pushed farther, inhibition will result.

6. Precipitants of Ca naturally cut short the rhythmic contractions.

7. Among salts that inhibit the movements otherwise than by precipitating Ca, and when present in small quantity, are $NaNO_2$, sodium benzoate, sodium salicylate, and the bile-salts.

My acknowledgments are due to Dr. Howell, who projected the research and shaped its course by many helpful suggestions.

PHYSIOLOGICAL STUDIES ON THE BLOOD OF ANIMALS DEPRIVED OF THE ADRENALS.

By ISAAC LEVIN.

[*From the Physiological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York.*]

THE extirpation of adrenal bodies performed now almost half a century ago by Brown-Séquard, and later by Tizzoni, Abelous and Langlois, Gourfein and others, showed conclusively that the function of those bodies is of vital importance to the organism, and that without them an animal cannot survive more than a few hours.

The symptoms accompanying the animal's death are so characteristic of acute intoxication, that most of the experimenters who studied the influence of the extirpation of the adrenal bodies on the organism came to the conclusion that the animals suffered from intoxication, and that the normal function of the adrenal bodies consists in neutralizing some poisons that would otherwise accumulate in the organism and injure it.

But with the exception of a few scattered statements that the blood of an animal deprived of its adrenal bodies is toxic (Abelous and Langlois [1] and Symonowicz [2]), this theory was hardly supported by experimental proof.

Some scientists found an increased quantity of neurin in patients suffering from Addison's disease, and further Marino-Zucci and Dutto[3] and Carbone [4] showed that neurin was much more toxic for animals deprived of their adrenal bodies than for normal ones. But these experiments are not sufficiently convincing to warrant the assertion that the adrenals normally neutralize neurin.

An entirely new turn was given to the question of the function of the adrenal bodies by the work done in the last six years. Oliver and Schäfer,[5] Symonowicz,[2] and many others showed that the aqueous ex-

[1] ABELOUS et LANGLOIS: Comptes rendus de la société de biologie, 1895, p. 334.
[2] SYMONOWICZ: Archiv für die gesammte Physiologie, lxiv, p. 97.
[3] MARINO-ZUCCI and DUTTO: Moleschott's Untersuchungen, xiv, 1892.
[4] CARBONE: Abstract in Allgemeine medicinische Centralzeitung, 1896, p. 846.
[5] OLIVER and SCHÄFER: Journal of physiology, xvi, xvii, xviii.

tract of the adrenal bodies contained a very active substance, the chemical nature of which is not yet fully determined, though the latest work of Abel [1] shows conclusively that the action of the suprarenal extract is due to epinephrin. This substance stimulates the vasomotor centre, the vagus, the respiratory centre and possibly the general muscle tonus. Cybulsky [2] showed that the blood taken from the adrenal vein has the same effect as the suprarenal extract, though not so pronounced. There can be no doubt that this active substance of the suprarenal extract is normally being constantly produced by the adrenal bodies.

As a result of this work the following conclusion was drawn as to the function of the adrenal bodies: these organs do not neutralize any toxic substances forming within the organism, but maintain the tonus of the vasomotor and respiratory centres and the general muscle tonus. In the absence of the internal secretion of the adrenal bodies, the organism succumbs from the depression of these nervous centres.

Now, it is apparent that this explanation does not in itself exclude the autointoxication theory, because the tonic action of the suprarenal extract may consist not in a direct influence on the centres, but in a neutralization of some substances formed within the organism, which would depress these centres. Marino-Zucci, for instance, showed that the action of the blood of an animal without adrenals is somewhat similar to the action of curare.

In order to decide the question, we must ascertain whether an animal deprived of its adrenal bodies is in a state of intoxication or not, and this is what I undertook in my research.

My *a priori* reasoning was, that if an animal without adrenals actually suffers from some form of intoxication, its blood will be likely to be abnormal, while if the state of the animal be due to the general nervous depression, we shall not find any change in the blood.

A secondary change of the blood due to the abnormal action of the vasomotor and respiratory centres could not be expected, as the animals after the operation do not develop any dyspnœa, and the circulation, though slower, is regular.

As the most sensitive reagent, I chose the influence of this blood on the blood-pressure of another normal animal. For control I ex-

[1] Abel: Zeitschrift für physiologische Chemie, xxviii, 1899.

[2] Cybulsky: Wiener medicinische Wochenschrift, 1896, 1 Feb.

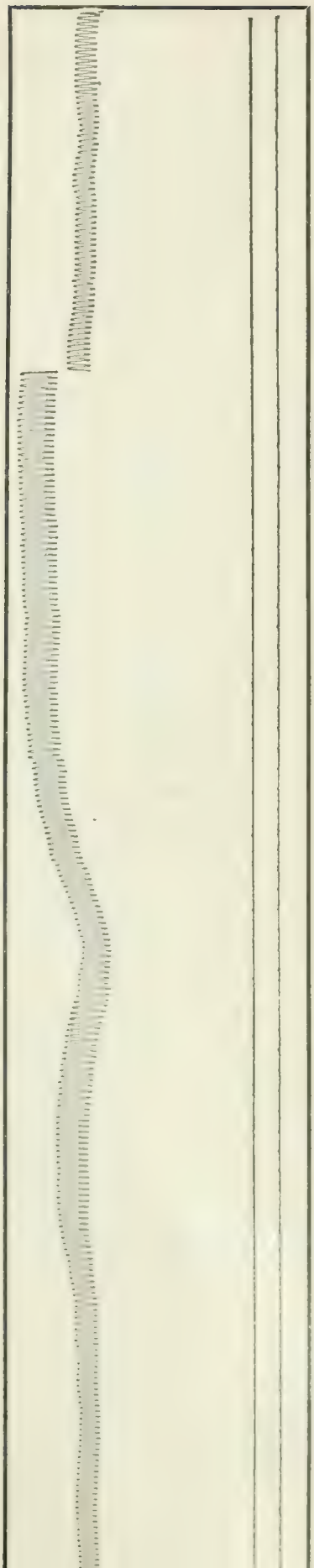


FIGURE 1.—The rise of blood-pressure after an intravenous injection of blood of animals deprived of the adrenals.

amined anew the influence of normal blood on the blood-pressure of another normal animal.

The animals used were dogs and cats. The following is the operative procedure: in the afternoon, both adrenal bodies were extirpated in the ordinary manner. Five hours later the animals were bled. At that time the animals felt quite well and ran about freely in the laboratory. The blood was defibrinated and kept on ice until the next afternoon, *i. e.*, for about seventeen hours. Then 50 c.c. of this blood was injected slowly into the jugular vein of a normal animal of the same species (dog or cat), the carotid artery of which was connected with an apparatus for recording the blood-pressure tracing.

For control normal animals were bled to death at the same hour of the night that the operated animals were bled. The blood was defibrinated and left on ice until the next afternoon, *i. e.*, for about seventeen hours, and then 50 c.c. of it was injected into the jugular vein of a normal animal and a blood-pressure tracing was taken.

I used the blood of eight dogs and four cats, of which the adrenal bodies were extirpated, and of six normal dogs and three cats. The results obtained were uniform. The injection of normal blood did not change in any way the blood-pressure tracing, while the injection of blood taken from an animal deprived of its adrenal bodies, gave a marked rise of the blood-pressure, which continued for from about two to three minutes after the injection. Several times

I noticed a double wave; the blood-pressure would begin to fall, and then rise again (Fig. 1). Very frequently also, it could be noticed that after the rise of blood-pressure, the character of the tracing would change, showing a weakening of the action of the heart, there being little difference between systole and diastole. In no case did I discover such change in the character of the tracing after injection of normal blood.

The conclusion I draw from these experiments is, that the blood taken from an animal deprived of its adrenal bodies, acts on another animal differently from normal blood, the former being apparently more active. This difference cannot be ascribed to operative shock, because, on the one hand, the operated animal seemed perfectly well during the bleeding, and on the other hand, the 50 c.c. of normal blood were taken from the whole quantity of blood of an animal bled to death. The second part of the blood was consequently taken at a time when the animal was under a severe shock, and still this blood had no effect on the blood-pressure. The difference must consequently be ascribed to the absence in one case of the adrenal bodies.

Now, the depression of the nerve centres in itself cannot explain the change in the constitution of the blood, and as I have shown this to be changed after the extirpation of the adrenal bodies, it must be admitted that the organism does not suffer from nervous depression alone, but that it undergoes some unfavorable change of its metabolism, some kind of an autointoxication. It may seem strange that the blood of an animal deprived of its adrenals and the suprarenal extract have both the same property of increasing the blood-pressure; but it must not be forgotten that this influence on the blood-pressure is only one property of a substance and may be identical in two substances, which are in every other respect antagonistic. The important fact remains that the blood of an animal without adrenals, contains something which acts on the blood-pressure and which does not exist in normal blood.

In conclusion, I desire to express my gratitude to Professors J. G. Curtis and F. S. Lee, in whose laboratory this work was done.

ON AN APPARENTLY NEW FORM OF MUSCULAR IRRITABILITY (CONTACT IRRITABILITY?) PRODUCED BY SOLUTIONS OF SALTS (PREFERABLY SODIUM SALTS) WHOSE ANIONS ARE LIABLE TO FORM INSOLUBLE CALCIUM COMPOUNDS.

By JACQUES LOEB.

[*From the Hull Physiological Laboratory of the University of Chicago.*]

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I. Introduction.

A SERIES of papers published from my laboratory has furnished the proof that the rhythmical contractions of striped muscles, the swimming bell of jelly-fish, the heart and the lymph hearts depend upon the presence of Na-ions in the surrounding solution. Calcium-ions have a tendency to diminish or inhibit the contractions altogether, although a small number of them must exist in the tissues in order to preserve contractility.[1] This point having been settled, I next tried whether the sodium ions bring about these effects directly or indirectly. I have not finished these researches so far as the rhythmical contractions of the muscle are concerned, but in pursuing this problem I have found a number of facts which show that certain salts can bring about effects indirectly by giving the muscle or nerve properties which they do not possess normally and which to my knowledge have not yet been described. If we put a fresh muscle (gastrocnemius) of a frog for a short time (*e. g.*, one to three minutes) into a solution of a sodium salt whose anion is liable to form insoluble calcium compounds (*e. g.*, NaFl, Na_2CO_3, Na_2HPO_4, sodium oxalate, sodium citrate, etc.), the muscle will as a rule not show any reaction except perhaps a slight shortening. But as soon as

[1] It is possible that certain other ions may act as a substitute for the Ca-ions for this purpose.

it is taken out of the solution and comes in contact with air, it goes into tetanus or performs a series of powerful contractions. The tetanus or the contractions cease at once and relaxation of the muscle occurs when the muscle is put back into the solution.

It was found that not only the change of contact from the above-mentioned solutions to air but also to a number of other media produce these contractions. What the nature of the stimulus in this case is I cannot say definitely. Provisionally I will assume that we are dealing with contact irritability and I will call the above-mentioned reaction of the muscle the contact reaction. It would seem as though the entrance of the anions of the above-mentioned solutions caused a change in the superficial layer of the muscle or its individual fibres, either by precipitating calcium or by otherwise altering the constitution of the protoplasm. This change is intensified by the increase in Na-ions in the same layer. In this condition the muscle is sensitive to the nature of the substance with which it comes in contact.

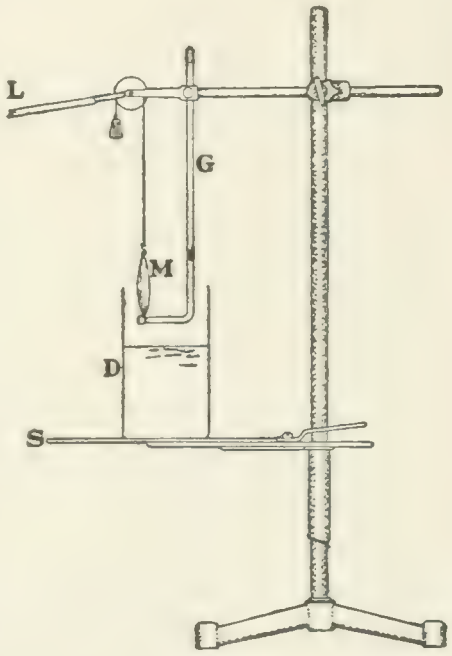


FIGURE 1. — Apparatus used to show the contact reactions of the muscle (M). The dish (D) contains the solution. By raising or lowering the support (S) the muscle can be submerged in the solution or brought into contact with air.

In these experiments one end of the gastrocnemius of a frog is tied to a glass rod, G (Fig. 1), and the other end is tied to the lever, L. A dish, D, containing the solution is raised from below when we wish to submerge the muscle, and is lowered when we wish to bring the muscle into contact with air.

In order to demonstrate the contact irritability I use a solution of 1 gram molecule of sodium fluoride or sodium citrate, etc., in about 8 or 10 litres. If the fresh gastrocnemius of a frog be put into such a solution for about one minute, the muscle will show a slight contraction when taken out of the solution. If the process be repeated a stronger contraction will follow when the muscle is removed, and after a series of submersions have occurred the muscle will give one or a series of powerful contractions every time it is taken out of the solution and brought into contact with air. After a certain time, which may be an hour or

more, and which varies according to the solution, the reaction becomes weaker and finally ceases.

If we use a stronger concentration than 1 gram molecule in 8 litres we get more powerful contractions, but the irritability of the muscle disappears sooner.

II. The Nature of the Solutions which produce Contact Irritability in Muscle.

Solutions of cane sugar or urea were unable to produce the contact reaction in muscle. I have tried these solutions in all concentrations from 0 to normal or even 2 *n*. A large number of electrolytes were then tested. None of the salts of Li, K, Ca, Mg, and NH_4 gave rise to the contact reaction. This statement is based upon experiments with LiCl, Li_2SO_4, Li_2CO_3, KCl, K-citrate, K-oxalate, $MgCl_2$, $MgSO_4$, NH_4Cl, $(NH_4)_2CO_3$, and ammonium citrate. The degree of dilution used was as a rule 1 gram-molecule in about 8 or 10 litres. In some instances stronger solutions were tried, but with the same negative result.

In my experiments on rhythmical contractions I have shown that the sodium ions have a specific rôle in the production of these contractions. It seemed also possible that they play such a rôle in the production of the contact irritability. But I found that $\frac{n}{8}$ or even stronger solutions of NaCl, NaBr, NaI, $NaNO_3$ did not bring about the contact irritability; neither did sodium acetate nor other salts whose anions form soluble calcium compounds.

But the sodium salts whose anions precipitate calcium promptly produce these reactions. NaFl, Na_2CO_3 Na_2HPO_4 sodium oxalate, sodium citrate,[1] sodium tartrate give the contact reaction in a dilution of 1 gram-molecule in 8 or 10 litres of water or even less. $NaHCO_3$ gives the reaction but requires a higher concentration, *e. g.*, 1 gram-molecule in 4 to 5 litres of water. If we put the muscle into a solution of Na_3PO_4 it goes at once into a powerful tetanus. This tetanus may be partly or wholly due to the high concentration of HO-ions in this solution. When a muscle goes into tetanus *in* a solution we cannot, as a rule, demonstrate the contact reaction. Thus I have never succeeded in producing contact reaction by a Na_3PO_4 solution. NaH_2PO_4 does not cause contact irritability, but this is in harmony with our general result.

[1] The citrates require an alkaline reaction for the precipitation of calcium. This condition is fulfilled in the fresh normal muscle.

The HO- and H-ions deserve special attention. In my experiments on rhythmical contractions I found that while they are not able to produce rhythmical contractions directly they accelerate the beginning of these contractions in the presence of Na-ions. In addition to such a catalytic action common to both the HO- and H-ions the former have another effect which they do not share with the H-ions. The muscle produces constantly H_2CO_3 and possibly other acids. These acids will increase the solubility of Ca-salts and increase the number of Ca-ions in the tissues. An addition of HO-ions will counteract this effect.

It is due to the presence of free HO-ions that solutions of Na-valerianate and Na-formate give rise to a slight degree of contact irritability in muscle, although calcium formate and calcium valerianate are soluble. If we diminish the alkalinity of a sodium formate and sodium valerianate solution by adding a small amount of free formic or valerianic acid (without, however, rendering the solution entirely neutral) they no longer produce the contact irritability in muscle. A small amount of alkali added to a NaCl solution may or may not produce a slight degree of contact irritability.

The solubility of $CaSO_4$ is comparatively high and we therefore cannot expect Na_2SO_4 to be very effective for the production of contact irritability. In solutions of 1 gram-molecule Na_2SO_4 in 10 litres or less, I sometimes got and sometimes failed to get the contact reaction. May it not be possible that the amount of free Ca-ions in the muscle of a frog varies at different periods of the year, and may not this fact account for the seasonal variation in the irritability of these animals? But if a Na_2SO_4 solution fail to produce contact irritability in a muscle an addition of some HO-ions will produce the desired effect. As a rule 4 c.c. $\frac{n}{10}$ LiHO or any other hydrate to 100 c.c. of the Na_2SO_4 solution is the optimum. We can produce the contact reaction also through the addition of a small amount of acid to the Na_2SO_4 solution, *c. g.*, 4 c.c. of $\frac{n}{10}$ HNO_3 (or any other inorganic acid) to 100 c.c. of the Na_2SO_4 solution. The effects are not so strong as if we add alkali.

The sulphates showed an exceptional behavior in still another direction. With one exception only sodium salts give rise to contact irritability and this exception is a sulphate, namely $(NH_4)_2SO_4$. It would almost seem that the sulphates have physiological effects aside from their effect upon calcium. This is in harmony with Miss Moore's experiments, in which she found that sulphates are as

capable of antagonizing the poisonous effects of a pure NaCl-solution as calcium salts.[1]

It should finally be mentioned that sodium butyrate, sodium succinate and sodium asparaginate did not produce the contact reaction.

Having thus proved that sodium salts, whose anions precipitate calcium give rise to contact irritability, it was to be expected that solutions of calcium salts would prevent or antagonize the contact reaction. I found that by adding a small amount of $CaCl_2$ to a Na-citrate solution the latter solution no longer produced the contact reaction. The addition of 1 c.c. of a 5 *n* $CaCl_2$ solution to 100 c.c. of an effective sodium citrate solution was sufficient to cause a muscle to lose its contact irritability at once. Only after a prolonged stay in a pure sodium citrate solution does the contact irritability return.

While all the facts thus seem to harmonize with the view that a decrease in the amount of Ca-ions in the tissues (and possibly an increase in the amount of Na-ions) is the essential condition for the production of the contact reaction, it is yet possible that the sodium salts whose anions form insoluble calcium compounds may have a specific effect upon other constituents of the protoplasm, *e. g.*, proteids.

III. On the Nature of the Apparent Contact Reaction.

The reaction which we have provisionally called the contact reaction appears when a muscle, after having been submerged in a sodium citrate or any of the other above-mentioned effective solutions, is brought into contact with air. In this change from solution to air a number of conditions change and it is now our task to determine which is the essential one.

As soon as the muscle is taken out of the solution and brought into air, more O_2 may diffuse into and more CO_2 may diffuse from the muscle. These two conditions have, however, nothing to do with the reaction. The experiments were repeated in an almost pure atmosphere of CO_2 instead of air and the contact reaction was as powerful as in air.

A second change is the sudden evaporation of water from the surface of the muscle upon its leaving the solution. The following experiment might suggest that this evaporation is the cause of the contact reaction. If we pack a muscle, that gives powerful contact

[1] Moore, A.: This journal, 1901, v, p. 87.

contractions, tightly in moist filter paper the reaction will not occur when the muscle is taken out of the solution, but will occur when the filter paper is removed. Nevertheless, evaporation has nothing to do with the reaction. We get the contact reaction quite as well in a moist chamber as in dry air. Furthermore we get the reaction if we bring the muscle directly from the sodium citrate or fluoride solution into oil, without exposing it to air. We can make this experiment in the following way. The lower half of the dish, D (Fig. 1), is filled with the effective sodium citrate solution, the upper half with oil (I used sperm and olive oil). The muscle is first brought into the sodium citrate solution and then, by lowering the support S, into the oil. Powerful contractions occur. Evaporation of water from the surface of the muscle is, therefore, not the cause of the contractions.

After this had been established it was to be expected that changes in temperature were not responsible for the contact reaction. Experiments in which the muscle was rapidly cooled and heated yielded only negative results.

The next possible cause to be considered was electricity. The fact that a change from the salt solution to a non-conductor (air, oil) caused contractions suggested the possibility that these contractions were in reality electrical break contractions, the muscle itself acting as a battery. The only fact which did not seem to accord with this explanation was the lack of a make contraction when the muscle was put into the solution. A number of experiments excluded the assumption that the contraction or tetanus of the muscle which occurs when it leaves the sodium citrate solution is due to a break shock. I connected the two opposite ends of the muscle by means of a thick copper wire. In this case the muscle contracted just as powerfully as before when taken out of the sodium citrate solution, although no break shock of any strength was possible. Another still more decisive fact was found. After the muscle had been treated for some time with a sodium citrate solution the break contraction could be produced by dipping the muscle for a short time, *e.g.*, thirty seconds, into a $\frac{n}{8}$ or $\frac{n}{4}$ solution of cane-sugar. As soon as the muscle was brought into contact with air, contractions occurred. The same was true for glycerine solutions. Both the sugar and the glycerine solution are non-conductors. The possibility of a mechanical stimulation as the cause of the contact reaction was next to be considered. As long as the muscle is in the solution each of its elements is under the hydrostatic pressure of the column of liquid above it. If we expose

the muscle to the air this pressure ceases. This might suggest the idea that a decrease of the hydrostatic pressure upon the muscle causes its contraction. The dipping of the muscle into the solution causes a relaxation of the contracted muscle and the inference should be drawn that an increase of the hydrostatic pressure causes relaxation. The following experiments prove the erroneousness of this view. The bottom of the dish was filled with a liquid of much higher specific gravity than the sodium citrate solution, *e. g.*, with chloroform, 2 *n* cane-sugar solution, or metallic mercury, and the sodium citrate solution was put carefully above the sugar solution or chloroform. The muscle was then brought from the sodium citrate solution into the sugar solution by raising the dish D (Fig. 1). In this case I noticed regularly one or more powerful contractions, although the hydrostatic pressure on the surface of the muscle was increased.

It thus seems to me that none of the known forms of muscular irritability suffices to explain the phenomena with which we are dealing. We have before us an apparently new form of muscular irritability, probably contact irritability.

Contact irritability is a very general form of irritability among plants and lower animals. I need only to remind the reader of the phenomena of stereotropism and of the fact that by mere contact-effects a polyp of a campanularia can be transformed into a stolon. But contact irritability certainly exists among certain cells of vertebrates, for example, the leucocytes. The nature of the body with which leucocytes come into contact determines whether or not they give off fibrin ferment and cause coagulation of the blood or other liquids which contain fibrinogen. How the nature of the contact can influence the leucocytes is still a mystery. One might think of surface tension phenomena or the formation of double electric layers at the surfaces in contact.

If the phenomena described in this paper were really contact phenomena, a further search should reveal that only a change of contact from certain bodies to other bodies can cause contractions of the muscle.

I have begun experiments in this direction, and have thus far found the following facts:

Contractions occur when the muscle passes :

From	To
Sodium citrate solutions	Air
Sodium fluoride solutions	CO_2
Sodium oxalate solutions	Oil
Sodium carbonate solutions	$2n$ sugar solution
Etc. (see above)	Glycerine
	Chloroform
	Toluol
	Mercury

Relaxation of the contracted muscle will occur when the muscle passes from any medium in the right column above to any medium in the left column.

After the muscle has been treated for some time with any of the efficient solutions (Na-citrate, etc.) the contractions are also produced when the muscle passes —

From $\frac{n}{8}$ or $\frac{n}{4}$ sugar solution to air.
From $\frac{n}{8}$ or $\frac{n}{4}$ glycerine to air.
From *any* salt solution to air.

A very interesting and theoretically important fact is that the muscle loses this peculiar form of irritability very soon when it remains in contact with air, oil, sugar solution, glycerine, or salt solutions different from those that produce this specific irritability. In LiCl or NaCl solutions the contact irritability is lost as fast, if not faster than in a sugar or glycerine solution. We can re-establish the irritability, however, if we put the muscle back into the sodium citrate solution for some time This fact, together with those mentioned before, suggests the following as the most probable explanation of the peculiar phenomena of contraction with which we have been dealing: the solutions which produce the contact irritability possess anions that are liable to form insoluble calcium compounds. They are all with one exception — $(NH_4)_2SO_4$ — Na-salts. Whatever the effects of these anions may be, the fact that in less than a minute the contact effects are noticeable indicates that only the surface layer of the muscle or what is less probable, the surface layer of each individual fibre is altered. It is impossible for the anions to migrate deeper into the muscle in so short a time. In the surface layer of the muscle or the individual fibres we have temporarily a diminution of Ca-ions. We have, then, a muscle whose surface-layer differs from that of an ordinary excised muscle. If this layer is once established the muscle contracts at any change from the media of the left column

of the above list to those of the right column. But it is obvious too that as soon as this change occurs the surface layer gradually undergoes an alteration, for example, in air, sugar solution, NaCl solution, etc. This change, in which the contact irritability is lost, occurs most rapidly in a $CaCl_2$ solution. This suggests the following possibility. The loss of contact irritability of the muscle in air or oil, etc., is due to the migration of Ca-ions from the interior of the fibre or the muscle to the surface, thus re-establishing approximately the original normal surface condition. If we then put the muscle back for a short time into a sodium citrate or sodium fluoride, etc., solution, a diminution of Ca-ions will again occur in the surface layers and the contact irritability will be re-established. As is to be expected the time the muscle remains in the solution is as important as the concentration of the solution. If we dip a muscle for a few seconds only into a sodium citrate solution (1 gram-molecule in 10 litres) the contact irritability cannot be produced, as there is not time for a large enough number of anions to diffuse into the muscle.

Still another fact harmonizes with our assumption. If we lift only a piece of the muscle out of the sodium citrate solution, not the whole muscle contracts, but only the individual fibres which come into contact with air. Similarly a more powerful contraction occurs when we lift the thick femur end of the gastrocnemius out of the solution than if we expose the thin tendon Achilles end to the air.

Finally it should be mentioned that the latent period is somewhat long in these experiments. I have not measured it yet exactly; but it may be a considerable fraction of a second, especially when the contact irritability is about to disappear. This somewhat long latent period would harmonize well with the assumption of contact phenomena.

Although I have spoken chiefly of the diminution of Ca-ions as the effect of the sodium fluoride and similar solutions, I wish to state that I consider it possible that these solutions may have other effects which play a rôle in these phenomena.

IV. The Effects of Sodium Fluoride and corresponding Solutions upon the Nerve.

If we try the experiments described above on curarized muscles we get little or no result. This would indicate that the contact reaction is not due to an effect of these solutions upon the *muscle* but upon the *nerve elements in the muscle*. There is a second possibility, namely

that curare, although it does not abolish the electrical irritability of muscle, may yet alter its substance enough to prevent the effects of contact stimuli, or prevent the formation of the hypothetical surface layer.

It may be said with certainty that sodium fluoride, sodium citrate, and the corresponding solutions act upon the nerve in a way altogether different from that in which they act upon muscle. If we put the nerve alone (without the muscle) into one of these solutions which contains 1 gram-molecule in about 10 litres, as a rule nothing will happen during the first five minutes. The removal of the nerve from the solution will not call forth a contraction of the muscle. After about five minutes the muscle will begin to twitch rhythmically, and very soon the muscle will shorten steadily until it reaches a high degree of tetanic contraction. This twitching continues as long as the nerve is in the solution. As soon as the nerve is taken out of the solution and exposed to the air the muscle relaxes more or less completely, and the twitchings become less numerous. As soon as the nerve is put back into the sodium citrate solution the contraction increases again and the twitchings become more powerful. This may be repeated very often. It is obvious that the nerve behaves in exactly the opposite way from the muscle. The latter contracts when taken out of the solution and exposed to the air, and relaxes when put back into the solution. If the nerve alone (without the muscle) be put into the solution, contractions of the muscle occur while the nerve is *in* the solution, and partial or complete relaxation is observed when the nerve is taken out.

These experiments on the nerve give one the impression that the sodium citrate and the solutions of the other sodium salts whose anions precipitate calcium stimulate the nerve chemically. Albert Mathews has recently found that weak solutions of sodium salts can cause contractions of the muscle when the nerve alone is put into the solution, while the salts of the other metals can only produce contractions when their osmotic pressure is considerably higher than that of the tissues. I have confined my experiments chiefly to those sodium salts whose anions precipitate calcium. But I think I can show definitely that these salts are not the direct stimulus that calls forth the contractions of the muscle, but play only an indirect rôle, inasmuch as they make the nerve more sensitive for another kind of stimulus, either a mechanical or a contact stimulus. When the nerve alone has been put into a sodium citrate solution (of 1 gram-molecule

in about 10 litres) and the muscle has begun to contract powerfully a gradual relaxation of the muscle is observed when the nerve is taken out of the solution and allowed to hang in the air. But at any time the contractions and the final tetanus of the muscle will begin again when the nerve is brought into contact *with any solid or liquid body*, no matter whether it is a conductor or a non-conductor. As soon as the contact ceases and the nerve is surrounded by air again on all sides the muscle gradually relaxes. This can be repeated quite often with the same result. Among the substances whose contact causes contraction I may mention hard rubber, glass, filter paper, varnished and unvarnished wood, bone, muscle, all kinds of metals. Among the liquids tried were oil, glycerine, sugar solutions and several salt solutions. It is thus obvious that in a sodium citrate solution two influences are united, first the effects of the citrate-ion which causes a modification or an increase in the irritability of the nerve, and second, the liquid character of the solution. The latter is the direct cause for the contraction.

Another point is of interest in this connection. The sodium citrate or sodium fluoride solution increases the electrical irritability of the nerve so that it can easily be stimulated by its own current of demarcation. This increase occurs regularly before the twitchings of the muscle begin.

In my experiments on artificial parthenogenesis in Chætopterus I found that there are two ways by which the unfertilized egg can be caused to develop — first, by certain ions (K, H), and second by causing the egg to lose water It follows from the facts of dissociation that a loss of water on the part of the egg must alter the proportion of ions in the egg. It thus becomes possible that the artificial parthenogenesis produced by the loss of water is in reality an ion-effect. In regard to the twitchings caused by putting the nerve into solutions Mathews has shown that two cases must be discriminated — first, the effect of specific ions, and second, the effect of loss of water. Any solution whose osmotic pressure is high enough can cause contractions if the nerve be put into it. Is it not possible that the loss of water in the nerve acts in the same way as the citrate or fluoride ions? The limited solubility of $CaSO_4$ would make this possible. I tried whether a nerve after having been put into a 2 *n* sugar solution long enough to cause muscular contractions, would show the above-mentioned mechanical or contact irritability. This was indeed the case. If such a nerve is taken out of the sugar solution and brought

into contact with solid bodies it gives rise to stronger contractions. But, as was to be expected, the nerve loses this irritability again when put into a ⅞ NaCl or Na-citrate solution. In such a solution water will enter the muscle and restore the original condition, and only later will the entrance of citrate ions show its effect.

It now remains to be seen how far these facts can throw light upon the heart-beat. The fact that a heart which has ceased to beat in a solution often begins to beat again when taken out of the solution reminds us of the contact reaction of muscle described above.

V. Summary.

1. Certain salt solutions (1 gram-molecule in 8 or 10 litres) bring about an apparently new form of irritability in muscles, which may be called provisionally contact irritability. A muscle that has been treated in this way will contract powerfully when it passes from the salt solution to air, CO_2, oil, sugar solution, etc., or from glycerine solutions, sugar solutions to air.

2. The salts whose solutions produce this form of irritability are (with one exception) sodium salts, whose anions are liable to precipitate calcium, namely:

Sodium fluoride	Na_2HPO_4	Sodium citrate
Sodium carbonate	Sodium oxalate	Sodium tartrate

3. If the nerve alone (without the muscle) be put into one of these salt solutions (1 gram-molecule in 8 or 10 litres) the muscle begins to twitch in about five minutes and finally goes into tetanus. If the nerve be taken out of the solutions the contraction ceases. Although this seems to indicate that the salts or their ions stimulate the nerve directly it can be shown that they only modify or increase the irritability of the nerve. For when the same nerve is brought into contact with any solid or liquid body (conductor or non-conductor) the contractions of the muscle will be resumed while they will gradually cease or diminish when the nerve is again surrounded by air on all sides.

4. The fact that certain ions are capable of bringing about forms of irritability in nerves and muscles which do not exist normally may perhaps furnish the explanation of a number of certain morbid phenomena (neuroses, hysteria) in which the motor and sensory reactions of the patient are modified.

ON THE FORCE OF CONTRACTION OF THE FROG'S GASTROCNEMIUS IN RIGOR, AND ON THE INFLUENCE OF "CHLORETONE" ON THAT PROCESS.

By N. M. STEVENS.

[*From the Physiological Laboratory of Bryn Mawr College.*]

IN the course of a series of experiments involving rigor of the frog's gastrocnemius, performed during the autumn of 1900, in the Physiological Laboratory of Bryn Mawr College, under the direction of Dr. Joseph W. Warren, the writer became impressed with the idea that much greater energy is exerted by a muscle undergoing rigor at a high than at a low temperature, and that the force of contraction varies directly as the temperature. A new series of experiments was therefore performed to test this impression, which was based only upon observation of the rise of the lever during rigor under various conditions.

The absolute energy exerted by the gastrocnemius in heat rigor has been measured by Hermann and Walker,[1] and compared with the energy of single contractions and of tetanus; but, so far as I know, no systematic work has hitherto been done to show the effect of temperature upon the energy of rigor contraction.

Method.

Apparatus. — The apparatus (Fig. 1) employed in these experiments with the exception of the spring, has been used in this laboratory for similar work since 1895, but has not been described.

It consists of a simple kymograph, a muscle-holder, and a reservoir of tin (an ordinary washboiler) supported on stands, beneath which gas burners may be placed as required.

The kymograph has a brass recording drum and spindle, A, set in the frame, B, and turned by a Seth Thomas eight-day marine clock sliding on a wooden track and fastened by a thumb-screw in any desired position. The minute and hour hands are replaced by two

[1] Hermann and Walker: Archiv für die gesammte Physiologie, 1871, iv, p. 182.

thin brass discs, one inch in diameter, and grooved for the waxed cord, R. The cone, C, composed of four circular plates of hard rubber, 1, 2, 3, and 4 inches in diameter respectively, cemented together on a central brass tube, slips up and down on its spindle and is fastened in position by the pin, D. By changing the cord from the minute to the hour wheel of the clock, and from one to another groove of the cone, adjusting the height of the cone for each change, the drum is made to revolve in 1, 2, 3, 4, 12, 24, 36, or 48 hours. The upper end of the spindle terminates in a circular

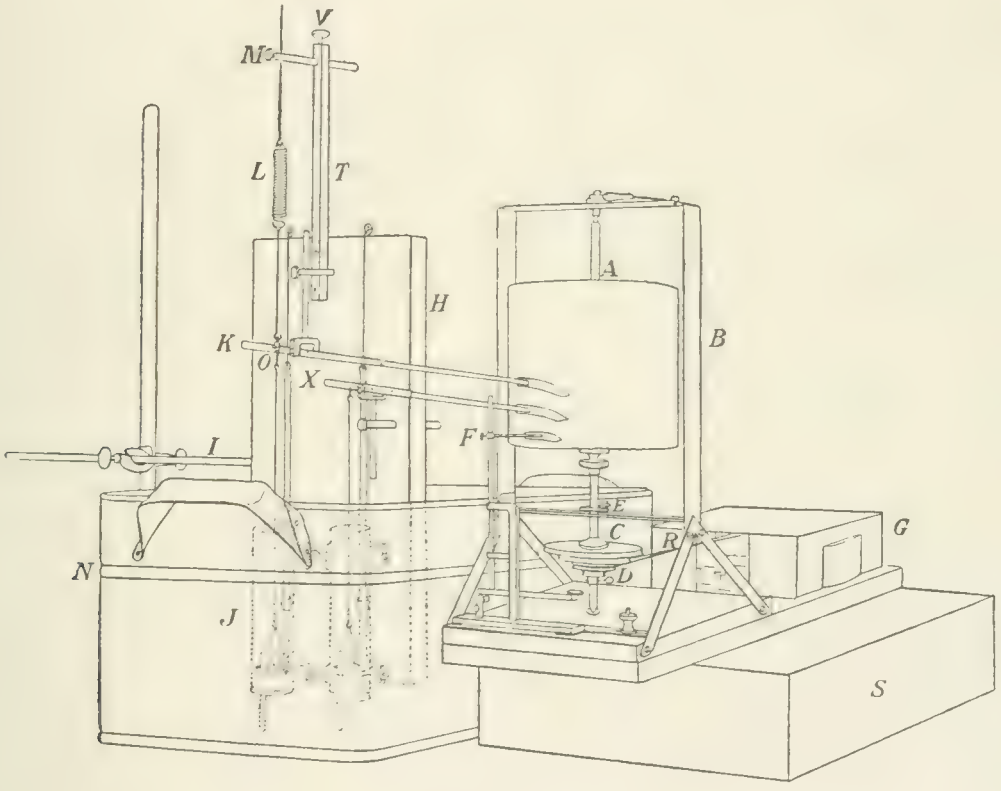


FIGURE 1.

plate, E, on which the drum is carried in the usual manner. The foundation of the structure is a hard wood board 20×48 cm. to which is fastened the clock track and another board, 20×21 cm. which carries the drum and marker. The latter part of the apparatus is removable for use in other combinations. The clock is protected from dust by a tin cover, G, through an opening in which the cord, R, passes. The kymograph rests on a stand made from a lecture room desk. In the sketch this stand has been omitted and the depth of the washboiler reduced about half.

The muscle holder, H, consists of a board, 17×26 cm., held in a

vertical position by the rod, I, which is clamped into a very firm stand, the base being filled with lead. The muscle cylinders, J, are of glass tubing, about 12 cm. long and 3.5 cm. inner diameter, supported against pieces of paraffined cork grooved to fit the tubing; they are held securely in place by tapes run through screw eyes and firmly tied over the cylinders. Each cylinder is fitted with a rubber stopper through which passes a short piece of paraffined doweling, held in place by a pin below the stopper; the femur of the muscle preparation is fastened into a hole in this rod by the pointed end of a hardwood toothpick.

The muscle is connected with the lever, K, by a light metal rod,[1] platinum below and steel above. This rod hooks into the tendon and into either the loop, O, or one of the holes in the lever.

The levers are of aluminium, pierced with holes at intervals of one fourth of an inch, for the attachment of the muscle-rod and weights, and lengthened by straws terminating in celluloid markers. The drawing (Fig. 1) shows the manner of suspending and adjusting the levers. The magnification is ten times, the long arms of the levers being 10 and 7.5 inches, the short arms 1 and ¾ inches, respectively.

For the attachment of the spring, L, a brass pillar, T, was screwed to the board. Near the top of the pillar is a brass rod, projecting at

[1] Paraffined or waxed threads were formerly used for such experiments, but certain peculiarities in some of the curves led me to think that even the most careful paraffining or waxing of the thread was not a sufficient safeguard against shrinkage. A quantity of linen thread was, therefore, thoroughly shrunken for immediate use, and later the matter was carefully investigated by setting up paraffined and waxed threads of linen, cotton, or silk, in liquid and in the cylinders used as moist chambers. In every case there was shrinkage for three or four hours, the temperature being raised, meanwhile, from 17° to 70° or thereabouts; upon cooling down to room temperature, the threads lengthened somewhat and shortened again slightly on heating, even after standing forty-eight hours, the final result being a shortening of 3.6 mm. for the length used in this apparatus, about 24 cm. A cotton thread shortened 5.3 mm. in three hours between 17° and 68°, lengthened 2.5 mm. during slow cooling to 17°, and again shortened 1 mm. on renewed warming about twenty hours after the first heating, giving a final shortening of 3.8 mm. Linen thread which had been soaked alternately in hot and cold water for several hours and then dried, showed no marked shrinkage when heated in the Ringer solution.

Vernon's statement (Journal of physiology, 1899, xxiv, p. 239) of the unreliability of thread for such work came to hand after the first tests were made, and his use of a metal chain was noted; but the rods, already in use for electrical experiments, seemed more convenient to handle, and were immediately substituted for the thread.

right angles to the surface of the board, and adjustable by the set screw, V. Through this rod passes a thick steel wire, to which is suspended a somewhat stiff steel spring of twenty-five turns. The spring is connected with the lever by another steel rod which hooks into a light brass support pinned through the hole in the lever with which the muscle is connected. The spring stretches just enough to let the lever make two distinct parallel lines on the drum for a difference of one gram in a scale pan hung from the loop, O. The rise of the lever for a weight of 200 grams is about half of the rise for the ordinary unresisted heat contraction of a muscle 30 mm. long.

A thermometer hung from a screw-eye in the top of the board, enters each cylinder, and pieces of thick rubber tubing hold the bulb away from the glass and near the muscle.

The reservoir is usually kept filled to the groove, N, the large amount of water, 30–35 litres, protecting the muscles from rapid changes of temperature when this is not desired. The amount of liquid used in the cylinders depends upon its character and upon the character of the experiment: at least enough must be used to cover the muscle and tendon.

Heat is supplied by an argand burner, with an adjustable arm, to this are added as many Bunsen burners as are required for rapid heating.

Preparation of the Muscles. — The frog was pithed, the muscle preparations removed as quickly and carefully as possible, and set up in the cylinders (Fig. 1). The spring, which was left loose while the connection between muscle and lever was being made, was now set at M to straighten the muscle. The liquid to be used was then poured into the cylinders, the levers adjusted to the drum, and heat applied to secure the desired temperature. The left gastrocnemius was used on the spring in each case, and the right as a control.

Every effort was made to avoid the use of any material which, in connection with the liquids employed, might injure the muscle. No metal except platinum was brought into contact with muscle or liquid; the rubber stoppers were thoroughly scalded and soaked for twenty-four hours before using; the platinum rods were sterilized in the flame before each experiment; and the cylinders were frequently scalded, especial care being taken in case of experiments continuing many hours.

In the first series of experiments a Ringer solution according to the following formula (Rusch) was used: —

Distilled water.	1000 c.c.
$NaHCO_3$	0.1 gm.
$CaCl_2$	0.1 gm.
KCl	0.075 gm.
NaCl	8.000 gm.

The results of ten experiments are given in Table I. In the temperature column, the first number given in each case shows the temperature at the beginning of the experiment; the second number, in heavy type, the temperature at the close of contraction

TABLE I.

Muscles in Ringer's solution.

No.	Length in mm.	Temperature C.	Time.	Force in grams.	Work in gm. mm.
			h. m.		
1	27	31.0°–**44.0°**	0 20	97.80	127.14
2	27	35.0°–**44.5°**	0 25	99.30	131.57
3	30	35.0°–**44.0°**	?	100.00	134.00
4	30	35.0°–**44.0°**	0 15	106.70	149.38
5	30	37.0°–**45.0°**	?	110.00	156.75
6	30	25.0°–**35.0°**	6 20	55.00	41.25
7	32	25.0°–**29.5°**	16 0	27.95	9.08
8	30	22.0°–**27.0°**	29 0	16.50	2.64
9	36	15.5°–**22.0°**	58 0	3.60	0.18
10	32	22.0°–**12.0°**	63 0	2.00±	0.05±

in Experiments 1 to 5, during the whole contraction in Experiments 6 to 8. In Experiment 9 the temperature varied considerably; it ran lower at night, but did not go above 22° C., and stood at that point during contraction. In Experiment 10 the culmination of contraction occurred at about 2 A. M., with the temperature somewhere between 20° and 12°, the latter temperature being observed at 8 A. M.

The pull of the muscle on the spring was determined [1] by ascertain-

[1] An attempt was made to establish a constant for the spring, but though C was found to be very nearly 40 grams to a millimetre, the method employed was thought to be more exact.

ing how many grams in a light scale-pan suspended from O (Fig. 1) were required to raise the lever from a base line drawn through the point where the lever began to rise to another line drawn through the highest point in the curve. The work done was calculated by the usual formula for an elastic resistance, $W = F \times \frac{1}{2}E$, E in this case being the resistance of the spring.

The muscles vary so much in thickness as well as in length, that the results can only be comparative, but as such they are sufficiently striking.

The question arose whether this decrease in energy of rigor contraction might not be due in part to changes induced in the muscle by long soaking in the Ringer solution. After some consideration of the various oils which have been used in muscle experiments, a light paraffin oil was selected as a medium which would certainly not be injurious, and would not be likely to be absorbed by the muscle, or to take anything from the muscle. The results of a second series of experiments, using the paraffin oil in place of the Ringer solution, are given in Table II: —

TABLE II.

Muscles in paraffin oil.

No.	Length in mm.	Temperature C.	Time.	Force in grams.	Work in gm. mm.
			h. m.		
11	28	23°–**44.7**°	0 40	81.00	85.86
12	31	24°–**44.3**°	0 35	127.35	213.31
13	30	**35.0**°	7 0	70.20	64.58
14	30	29°–**33.0**°	15 0	44.00	26.40
15	29	22°–**27.0**°	30 0	31.70	18.79
16	34	17°–**22.0**°	53 0	3.60	0.18

Experiment 11 of this series was a very slender muscle, containing much less substance than Experiments 1 or 2 of Table I, and the muscle of Experiment 13 was very much stouter than that of Experiment 6, the corresponding muscle of Table I.

The curves shown in Fig. 2 were traced directly from the drum record of Experiment 2, those in Fig. 3 from Experiment 12. Both are characteristic curves, but the peculiarities observed in comparing

one with the other are not characteristic of experiments with either the Ringer solution or the oil. The counterpart of any variation in

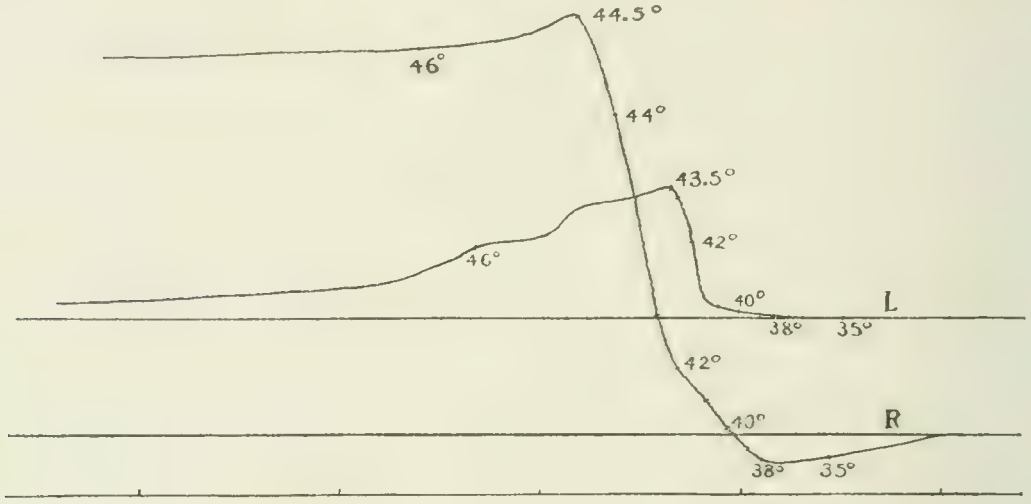


FIGURE 2.

form of curve or of temperature limits may be found in the records of experiments with the other liquid. Comparison of the two tables indicates that the results obtained are independent of the liquids used.

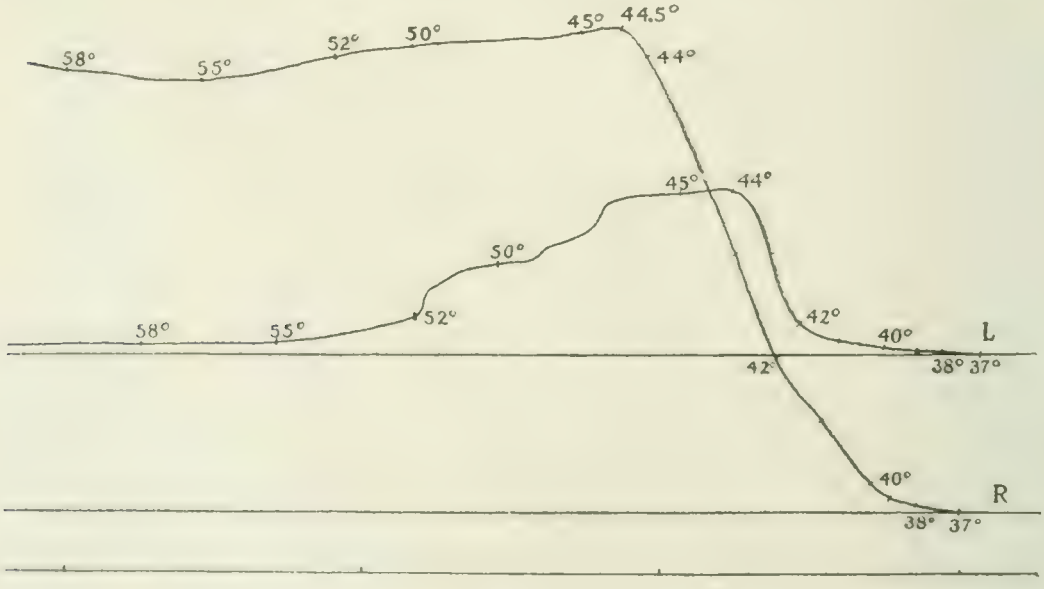


FIGURE 3.

Secondary heat contractions were obtained in the control muscles between 50° and 58°, and between 60° and 63°; but in no case did these contractions exert any additional force on the spring.

The temperature limits in these experiments are higher than those

given by Brodie and Richardson,[1] for corresponding heat contractions in the sartorius of the frog, but agree with those given by Latimer[2] for the gastrocnemius.

This difference is probably due mainly to the greater thickness of the gastrocnemius, but the statement of Moriggia[3] that the proteids of winter frogs coagulate less readily may have some bearing on the point.

For comparison with Walker's results, a few experiments were performed, in which one muscle of a pair pulled on a spring, during heat contraction, and the other was "afterloaded" with two hundred grams.

The only change in the apparatus for these experiments was the addition of a firm support for the lever X, — a heavy brass rod, bent at right angles and passed through an adjuster projecting from a metal plate which could be fastened to the board, in any desired position, with an ordinary clamp.

The following table is a record of four such experiments, the work being completed by the usual formulæ, $W = F \times \frac{1}{2} E$, and $W = F \times D$.

TABLE III.

No.	Length in mm.	Spring.		Load of 200 gms. Work in gm. mm.
		Force in grams.	Work in gm. mm.	
1	30.0	142.3	256.14	130
2	29.0	138.9	239.60	90
3	27.5	104.0	140.40	30
4	27.0	98.1	122.60	10.5

Influence of "Chloretone" on Rigor Contraction.

The so-called "chloretone" of commerce (Acetonchloroform = trichlor tertiary butyl alcohol) having been found useful for several purposes in this laboratory, it was suggested that its direct action on frog's muscle might prove interesting.

[1] Brodie, T. G., and S. W. Richardson: Philosophical transactions, cxci, p. 127.

[2] Latimer, Caroline W.: This journal, 1898, ii, p. 29.

[3] Moriggia: Moleschott's Untersuchungen, 1892, xiv, p. 386.

The apparatus used was the same as in the preceding experiments. The chloretone was dissolved in the same Ringer solution, and 150 c.c. were used for each muscle. The temperature of the water in the reservoir was kept at 30° and that of the chloretone solution, when it was poured into the cylinders, was about 20°. The results were

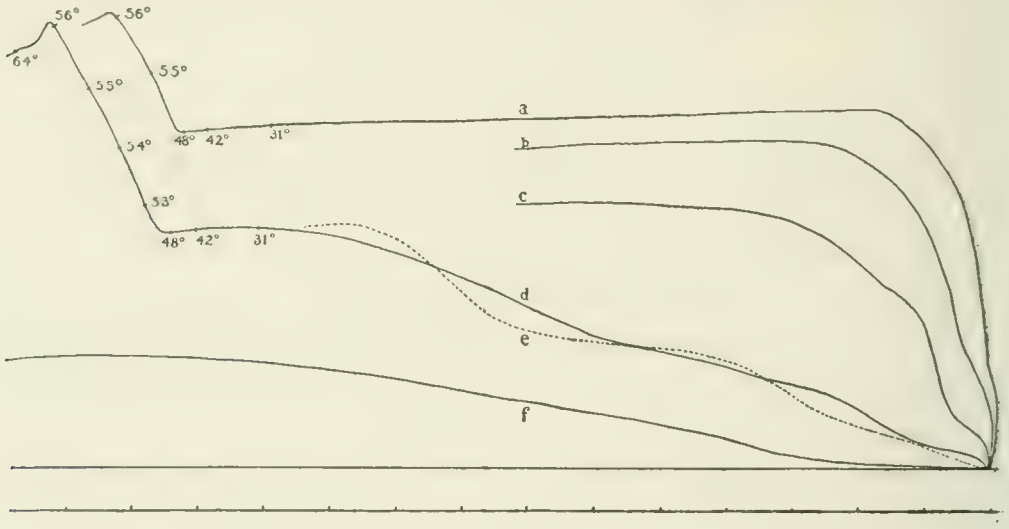


FIGURE 4.

practically the same with the reservoir water at room temperature, but the time required for the experiments was considerably longer.

The curves of contraction made by three pairs of muscles in chloretone solutions of different degrees of concentration, 0.1 per cent to 0.5 per cent of chloretone crystals in Ringer solution, are shown in Fig. 4, and further details are given in Table IV.

TABLE IV.

Muscles in Chloretone.

	Length in mm.	Per cent of chloretone.	Time.	Per cent of shortening.
			h. m.	
a	29	0.5	0 58	37.2
b	29	0.4	1 28	34.1
c	26	0.3	3 0	30.7
d	26	0.2	5 40	27.7
e	26	0.2	4 50	28.0
f	26	0.1	6 30	12.7

The levers were adjusted to the drum before the solutions were poured into the cylinders, and all of the muscles except f, at once began to shorten more or less rapidly according to the concentration of the solution; f, however, began to contract only after eight and one-half hours.

The rate and percentage of shortening decrease with the strength of the solution, much as in rigor at different temperatures from 45° to 20°, but with greater regularity and certainty.

On applying heat to muscles that have undergone chloretone contraction, no change is observed between 37° and 45°, but secondary heat contractions, perfectly comparable with those of muscles which have undergone rigor mortis or rigor caloris, occur between 50° and 60°. The height of these contractions depends on that of the previous contraction, as is shown in Fig. 4. The secondary contraction, which is evidently a combination of the second and third heat contraction described by Brodie and Richardson for the sartorius, is enough greater in one muscle of a pair, treated with a 0.2 per cent chloretone solution, than that of the other in a 0.05 per cent solution, to make the entire shortening in the two muscles approximately the same.

The same phenomena are observed in muscles which have undergone rigor mortis at different temperatures: a large secondary heat contraction follows a small rigor mortis contraction, and *vice versa*.

In both cases, the first heat contraction, due, according to von Fürth[1] and to Brodie and Richardson, to coagulation of soluble myogen fibrin, is cut out, in one case by the chloretone contraction, in the other by rigor mortis; but the contractions, due according to the same authors, to coagulation of myosin and myogen, and to contraction of connective tissue occur as usual, but with the variations described above. It would seem that there must be a different molecular disposition of the coagulated substance in a slow primary rigor contraction which makes a greater secondary contraction mechanically possible.

The effect of chloretone in 0.5 per cent solution seems to be quite similar to that described by Kühne[2] for potassium sulphocyanide (Rhodankalium), but its action appears to be definitely selective, precipitating soluble myogen fibrin, and not myosin or myogen (von Fürth).

[1] Von Fürth: Archiv für experimentelle Pathologie und Pharmakologie, 1895, xxxvi, p. 231, and 1896, xxxvii, p. 389.

[2] Kühne: Archiv für Physiologie, 1859, p. 634.

The action of chloretone on the proteid extracts of muscle should now be ascertained: if it should be shown that it precipitates soluble myogen fibrin in solution, and not myosin or myogen, we should have positive evidence that these proteids exist as such in the living muscle, and that the phenomena of heat rigor are due to fractional coagulation or precipitation of the same proteids.

For comparison with the chloretone contractions, a few experiments were performed with chloroform water (saturated solution), and with potassium sulphocyanide (KSCN), the Rhodankalium of Kühne.

The curves obtained with chloroform water were practically identical with those of chloretone, 0.5 per cent solution. This fact supports the theory that the action of chloretone as an anæsthetic is due to separation of chloroform in the tissues.

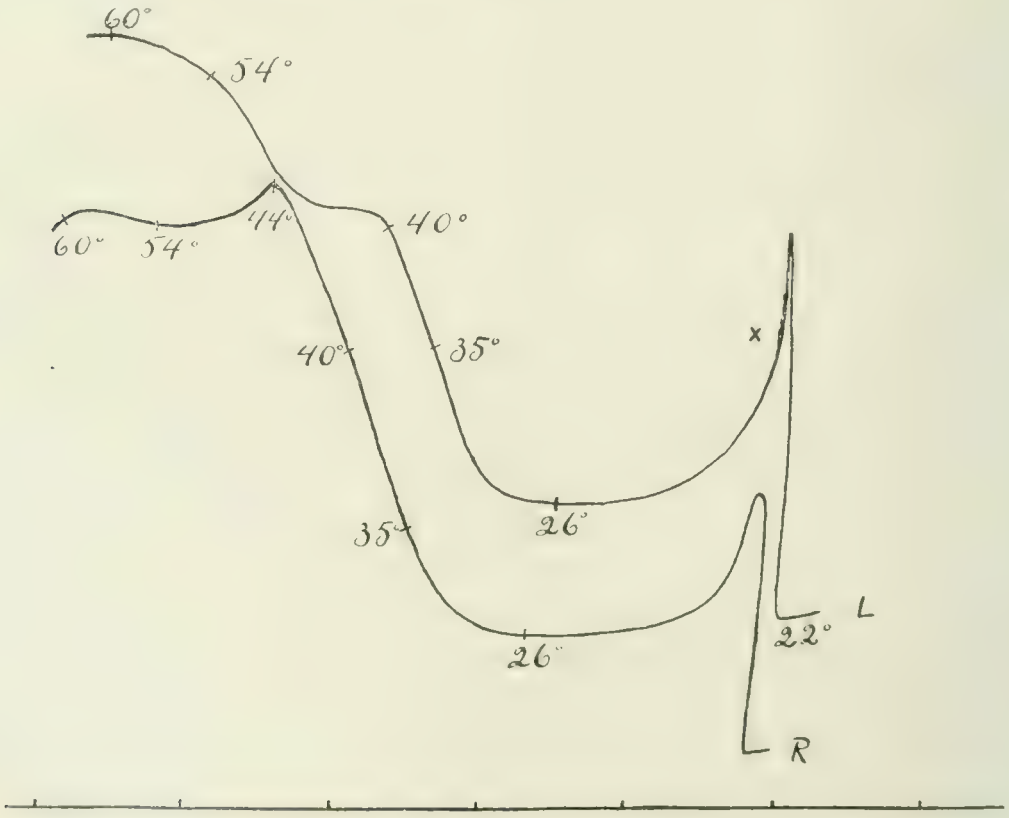


FIGURE 5.

The curves produced by the action of the potassium sulphocyanide showed unexpected peculiarities. At first, a 1 per cent solution as mentioned by various authors, was used, and later $\frac{1}{2}$ per cent and 2 per cent solutions.

In every case the lever rose rapidly to a certain variable height and then sank, the amount of rise and fall varying with the strength

of the solution. After the lever ceased to fall, heat was applied and more or less irregular primary and secondary heat contractions occurred.

Fig. 5 shows the curves made by a pair of muscles, L in a 2 per cent solution, R in a ½ per cent solution. At X the muscle gave several slight twitches, showing that it had not been deprived of irritability by the sulphocyanide. The whole appearance of the initial contraction suggested a sort of tetanus. One pair was taken down after an hour without heating, and the muscles appeared perfectly fresh: another pair was left set up for several hours at room temperature: within an hour from the beginning of the experiment, both of these muscles made slight contractions and then gradually lengthened; after forty-one hours both muscles made large contractions on heating to between 50° and 60°.

Four different samples of the chemical, all supposed to be "C. P.," were tried with the same result.

The results of these last experiments, compared with those performed with chloretone tend to confirm the impression that chloretone may prove valuable in the study of proteid coagulation in its relation to rigor contraction.

Conclusions.

1. The energy of rigor contraction decreases rapidly with decrease in temperature, being very slight at 20° C. or below.

2. The energy of secondary heat contractions (50°–63°) in the gastrocnemius is less than that of ordinary rigor contraction at 20 C.: it is not measureable by the spring used in these experiments, even when the muscle has been stretched to its original length before the temperature of secondary heat contractions is reached.

3. There is a certain degree of parallelism between temperature, rate, amount, and energy of rigor contraction.

4. A gastrocnemius muscle, undergoing heat rigor, does more work when it contracts against a spring than when it is "after-loaded" (200 grams.)

5. The fact that complete rigor at 20° C. entirely cuts out the first heat contraction (37°–45°), but not the secondary heat contractions (50°–63°) indicates that rigor mortis of frog's muscle involves coagulation of soluble myogen fibrin (v. Fürth), but not of myosin or myogen.

6. The fact that immersion of a muscle in chloretone solution

(0.1–0.5 per cent) produces a contraction which cuts out the first heat contraction (37°–45°), but not the secondary contractions (50°–63°) indicates that the result of its action is coagulation of soluble myogen fibrin, but not of myosin or myogen (v. Fürth).

7. A slight contraction produced by weak chloretone solutions (0.1–0.3 per cent) is compensated by a proportionately greater secondary heat contraction, — as in the case of slight rigor mortis contractions (Brodie and Richardson), — indicating a different molecular disposition of the coagulated proteid in a slow primary contraction, making a greater secondary heat contraction mechanically possible.

8. The action of chloretone is very definite and constant, as compared with that of potassium sulphocyanide.

In conclusion, I desire to express my thanks to Dr. Joseph W. Warren for suggestions and criticism.

CHEMICAL STUDIES OF OSSEOMUCOID, WITH DETERMINATIONS OF THE HEAT OF COMBUSTION OF SOME CONNECTIVE TISSUE GLUCOPROTEIDS.

By P. B. HAWK and WILLIAM J. GIES.

[*From the Laboratory of Physiological Chemistry, of Columbia University, at the College of Physicians and Surgeons, New York.*]

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I. Preparation of Osseomucoid.[1]

HISTORICAL.

IT seems to have become generally accepted that osseous tissue does not contain glucoproteid. A study of the statements in the recent text-books, regarding the composition of bone, reveals the fact that either the existence of mucoid in bone structure proper is directly denied or else that nothing whatever is said as to its possible presence. The marrow of bone, however, has repeatedly been said to contain mucin, although reference to the sources of the information usually given in this connection shows that very little

[1] Gies: Proceedings of the American Physiological Society (New Haven meeting, December, 1899); This journal, 1900, iii, p. vii. Also, Gies: Proceedings of the American Association for the Advancement of Science (New York meeting, June, 1900), 1900, p. 131. See foot-note, p. 402, for reference to subsequent report.

work has been done to ascertain the facts, and that the results of that work are anything but conclusive.

Neumeister[1] states, in this connection, that "neither mucin, nor any body belonging to the glucoproteids, has ever been detected in osseous tissue, although fibrous connective tissue and cartilage do contain such substance." Referring to ossein, prepared in the usual manner, Gautier[2] writes: "It does not yield glucose (reducing substance) after prolonged boiling in dilute acid." "The absence of mucin in compact bone is noteworthy," says Halliburton,[3] "showing that the ground substance is entirely replaced by calcareous matter. Marrow, however, yields mucin." Hammarsten[4] gives considerable attention to the composition of bone, but ignores this phase of the subject altogether.[5]

Morochowetz,[6] in 1876, called attention to the fact that the so-called "chondrin" or "cartilage jelly" of the older writers was in reality a mixture of substances. Morochowetz stated that it consisted of gelatin and mucin. Drechsel,[7] referring a few years ago to Morochowetz's deductions in this regard, wrote as follows: "If chondrin is in reality gelatin + mucin, the transformation of cartilage into true bone is all the more easily comprehended, for in that case such development would consist essentially in only the elimination of the mucigenous constituent." The deposition of inorganic matter in addition is, of course, to be understood.

For years it was said that cartilage would yield chondrin, but that true bone would not. The views of Hofmann[8] are representative of those held for a long time. He stated that "chondrin may be obtained from bone *before* ossification, but ossified bone yields only gelatin." At another place Hofmann writes:[9] "Embryonic bones contain no collagen but do contain chondrigen, which is not transformed into the first-named, but before ossification is displaced by it. Completely calcified bone does not contain even a trace of

[1] Neumeister: Lehrbuch der physiologischen Chemie, 1897, p. 453.

[2] Gautier: Leçons de chimie biologique normale et pathologique, 1897, p. 108.

[3] Halliburton: Schäfer's Text-book of Physiology, 1898, i, p. 111.

[4] Hammarsten: Lehrbuch der physiologischen Chemie, 1899, p. 326 *et seq.*

[5] See note, p. 400.

[6] Morochowetz: Jahresbericht über die Fortschritte der Thierchemie, 1877, p. 37.

[7] Drechsel: Hermann's Handbuch der Physiologie, 1883, Bd. v, Th. 1, p. 598.

[8] Hofmann: Lehrbuch der Zoochemie, 1875–78, p. 25.

[9] Hofmann: Ibid., p. 32.

chondrigen." Mörner[1] finally showed that cartilage contains chondromucoid ("mucin"), chondroitin sulphuric acid, collagen and albumoid (elastin?), and that chondrin is composed of the first two of these and gelatin.

Bone marrow. — Hoyer's[2] histological studies led him to assume that the ground substance of bone marrow is a loose, soft, mucous tissue. He did nothing in a chemical way to substantiate this view. Rustizky,[3] some time later, working with Rexlinghausen and under Hoppe-Seyler's direction, pointed out the incorrectness of this inference of Hoyer's, but, nevertheless, claimed to have shown the presence of a water-soluble mucin in the marrow of the bones of the rabbit. It was found to be absent from the marrow of the ox. Bone marrow from other animals was not examined.

It may reasonably be doubted, however, whether Rustizky's work is entirely reliable, for his deductions were based solely on the reduction test with alkaline copper solution after acid-decomposition of acetic acid precipitates, and no assurance was given that reducing substances were removed before the treatment with acid was begun, nor, indeed, that the precipitate itself had any proteid qualities other than precipitability with acetic acid. Further, the positive result with the rabbit tissue is referred by Rustizky and those who quote him, to marrow alone, although in Rustizky's experiments, after the adherent muscle had been removed, the whole bone, including the periosteum, was finely broken up in a mortar and the *mixture* extracted for mucin. It might with good reason, therefore, be assumed that any mucin really detected came from the periosteum, or the compact portion, instead of the marrow of the bones of the rabbit, and that a negative result was obtained with the ox marrow because the latter had been previously removed from the bone and, as Rustizky states, treated separately.

The question should still be regarded as an open one. Since Rustizky's time no results have been reported bearing on this subject. The author hopes to complete, in the near future, more definite experiments in this connection.

Compact Bone. — The experimental results repeatedly given as authority for the statement that mature, compact bone does not contain mucin have led to equally uncertain conclusions. No particularly

[1] C. Th. Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

[2] Hoyer: Centralblatt für die medicinischen Wissenschaften, 1869, p. 257.

[3] Rustizky: *Ibid.*, 1872, p. 561.

chemical investigations seem to have been made in this connection until a few years ago. Von Ebner[1] had shown that the decussating fibres of Sharpey are similar to those in fibrous connective tissue in general, and that they are not calcified, but that the calcareous deposit in bone is confined to the interfibrillar areas. These observations led Young[2] to investigate the question whether the matrix, in which the fibres of the bone structure are embedded, "is completely calcified or not." He concluded that this question could be most readily solved by ascertaining whether mucin, "the most abundant constituent of the uncalcified matrix or ground substance of connective tissue, is present or absent." Working under Halliburton's superintendence, Young failed to extract from bone with lime water or dilute baryta water any substance that could be precipitated with acetic acid. He concluded, because of this seeming absence of glucoproteid from compact bone, that, "in the process of ossification, the connective tissue matrix is apparently completely calcified." Young's results would imply the absence, from bone, not only of mucin but of chondromucoid as well, deductions which remained undisputed, so far as the author knows, until this work was begun.

Young's result and his general conclusion did not seem to harmonize with several well-known facts. Mörner's[3] researches, for example, on the proteids of cartilage, which were published in detail several years before Young's results were announced, showed that chondromucoid is present in relatively large quantity in that tissue, and of course suggested, further, that bone derived from cartilage contains a chondromucoid residue.

Practically all forms of uncalcified fibrous tissue from which the intercalated material has not entirely disappeared are known to contain mucin; yet bone, according to Young, would be regarded as an exception, although its large quantity of ground substance holds "bone corpuscles" in great number, and it contains circumferential, decussating and perforating fibres, as well as the fibrillar tissue of the Haversian canals and the fibrous structures among the "systems."

Since bone is formed in all cases by an ossification of connective tissue, and as collagen and other proteids are among the substances regularly contained in bone, it seems natural to suppose that during the developmental changes some of the connective tissue glucoproteid

[1] Von Ebner: Archiv für mikroskopische Anatomie, 1887, xxix, p. 213.

[2] Young: The journal of physiology (English), 1892, xiii, p. 803.

[3] C. Th. Mörner: *Loc. cit.*

would remain with the other organic substances. Furthermore, if glucoproteid has any definite function to perform in the connective tissues, if its presence there signifies anything, there is certainly reason to believe that it plays some part, however obscure, in bone metabolism, also. The organic constituents already identified in bone, or, let us say, the usual connective tissue elements which remain in bone after ossification is complete, are, according to Halliburton, "collagen, small quantities of elastin from the lining of the lacunae and canaliculi, proteids and nuclein from the cells, and a small quantity of fat even after the removal of all the marrow." [1] Why not mucin or chondromucoid? Surely, unless the ground substance of the antecedent tissue is *entirely* removed as impregnation with inorganic matter proceeds and permanently replaced in the mature bone — and there is no histological evidence of any such fact — mucoid substance ought to be separable, in small proportion at least, from osseous tissue.

Upon referring to Young's paper the author was impressed with the inadequacy of the method which had led to only negative results and conclusions. Young treated hard, compact bone, either in the form of fine shavings or in powder, for from three to five days with a "*large excess* of lime water or dilute baryta water." Just what the "large excess" was intended to accomplish it is hard to surmise; for, on the assumption that probably at most only a very small proportion of mucin could be present in bone, subsequent precipitation would be favored if the extract were kept concentrated. Even finely divided tendon is usually treated with only 2 to 4 c.c. of half saturated lime water for every gram of tissue extracted, when easy separation of its glucoproteid is desired, and tendon probably contains relatively as much mucin as any other form of connective tissue. In Young's experiments as much as 100 c.c. of the dilute alkali was taken for each gram of substance extracted.

Another defect in Young's work that the author regrets to call attention to was the use of too small quantities of bone. In one experiment only 2.5 grams of bone powder were used; in the best of them only 11 grams were taken. According to Halliburton the normal adult connective tissues contain 0.5 to 0.8 per cent of mucin.[2]

[1] HALLIBURTON: *Loc. cit.* It is in connection with this statement that Halliburton accepts the results of the work of Rustizky and Young, with the comment already quoted.

[2] HALLIBURTON: Text-book of chemical physiology and pathology, 1891, p. 478.

The largest amount of mucin Halliburton and Stevenson obtained in their quantitative work was 1.02 per cent — from skin.[1] From the human Achilles tendon the largest amount obtained by them was 0.77 per cent. Now, if we assume for the moment that bone might contain as much mucin as was found in the skin analyzed by Halliburton and Stevenson — roughly 1 per cent — an assumption far too liberal, then the 2.5 grams of bone employed in one of Young's experiments might have yielded 0.025 gram of mucin in the 100 c.c. of dilute alkali used, or the 11 grams in the best of Young's experiments might have given 0.11 gram in 500 c.c. of solution. But these amounts are the greatest which could have been assumed to occur in bone and certainly it would have been extremely difficult, if not impossible, to precipitate smaller quantities than these from extracts purposely made so dilute. Solutions of pure mucin containing approximately these minute amounts of the proteid may yield flocculent precipitates with concentrated acetic acid after standing some time,[2] but tissue extracts, holding other dissolved proteids and saline matters, act differently.

As has just been indicated, the very small quantities of bone powder or shavings, used in Young's experiments, were treated for several days with a large excess of lime or baryta water. At the end of that time, varying amounts of acetic acid were added and, to use Young's own phrase, "no precipitate came down in any case." Nothing is said about turbidity, yet traces of mucin under these conditions certainly could hardly have caused more than cloudiness.

The chief objection, however, to the method Young employed was the direct application of dilute lime or baryta water to a dense compact tissue, thoroughly impregnated with salts which for the most part are insoluble in such medium. It is not difficult to understand how, in the case of the femur, for example, the stone-like structure of the compact portion, composed as it is largely of tribasic earthy phosphates, imposed a serious obstacle to the usual action of lime water on contained mucoid substance, and therefore it is natural to assume that for this reason, if for no other, no mucin was detectable in Young's experiments. Minute division of the dense tissue in this instance could hardly make the conditions more favorable for extrac-

[1] Halliburton and Stevenson: *Ibid.*, p. 478.

[2] This can occur only when the mucin has been dissolved in a very small quantity of dilute alkali. The salts formed on acidification tend to keep mucin in solution.

tion. The proportion of inorganic matter, and its influence against extraction of mucoid, would naturally remain almost the same in every particle, however small.

These obvious defects in the methods heretofore employed led the present writer to investigate this very simple problem in a way which seemed more favorable to the separation of mucoid. The several difficulties just alluded to have been overcome by very ordinary means, and a substance has been prepared from bone having all the general characters of the glucoproteids.[1]

METHOD OF PREPARATION.

In a few preliminary experiments, merely to test the objections here raised against Young's methods, but with no expectation of more definite results than he obtained, the author used 200–250 grams of powdered femur — made from only the compact portion of the shaft, which had previously been thoroughly scraped with a scalpel for the removal of all superficial connective tissue. These quantities were much larger than Young's. The femur powder was extracted for several days with just enough half-saturated lime water to cover it. On several occasions a very faint turbidity was obtained upon adding to the filtered extract 5 per cent acetic acid or 0.2 per cent hydrochloric acid until the reaction was distinctly acid. Even after standing a long time, the turbidity remained diffuse, and, as in Young's experiments, borrowing his phrase again, "no precipitate came down." But the turbidity was encouraging.

The author next proceeded to remove the salts from the bone as a necessary preliminary to extraction in dilute alkali, and by the following method succeeded in obtaining a surprisingly large yield of glucoproteid from both the femur and the rib of the ox.

The fresh bones, just after removal from the animals, were freed as thoroughly as possible from adherent muscle and connective tissue. In order to prevent putrefactive complications, the marrow, in the case of the femur, was completely cleaned out and the bones then placed in running water for twenty-four hours. At the end of that

[1] The terms mucin, mucoid, and chondromucoid have been used here to refer to connective tissue glucoproteid. Recent researches seem to indicate that the particular substances to which these names have been applied are not as different chemically as had been supposed. See CUTTER and GIES: Proceedings of the American Physiological Society; This journal, 1900, iii, p. vi. Also PANZER: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 363; and LEVENE: *Ibid.*, 1901, xxxi, p. 395.

time the closely adherent connective tissue was somewhat swollen and could easily be completely scraped from the bones with an ordinary heavy scalpel. The inside of the shaft of the femur was again thoroughly swabbed. After this had been accomplished the bones were kept in 0.2–0.5 per cent hydrochloric acid. In the course of a few hours the dilute acid took out the inorganic matter from the surface of the bones just as satisfactorily, although not so rapidly, as much stronger acid could have done. It was better adapted for the purpose, also, because there was no special danger that transformation of mucoid would result from its use, — a fact of which there could be little doubt, because the acidity of the fluid in contact with the bones was constantly diminishing by reaction with the earthy compounds.[1]

After this treatment the bones were scraped twice daily with a stout, well-sharpened scalpel. The superficial decalcified layer was thus easily removed in long, narrow, thin, elastic shavings, exceedingly soft and pliable. The dilute acid was completely renewed after each scraping.[2] The ossein obtained in the first two scrapings was thrown away, for fear it was contaminated with minute particles

[1] This fact was observed repeatedly. The following results of one experiment in this connection show how rapid is the decrease of total acidity. In several preliminary titrations 100 c.c. of a special 0.5 per cent HCl solution was found to be exactly neutralized by 38.2 c.c. of a convenient dilute solution of ammonia; congo red was used as the indicator. A perfectly fresh femur of the usual size, after it had been thoroughly cleaned, was placed in 1000 c.c. of this particular solution of 0.5 per cent HCl. At intervals, after the fluid had been thoroughly stirred, total acidity was determined, with the same alkaline solution, in portions that had been boiled, for a few minutes, for elimination of carbon dioxide:

5.45 P. M. (femur first placed in acid) : 100 c.c. neutralized by 38.2 c.c. NH_4OH.
8.00 P. M. : 100 c.c. neutralized by 18.2 c.c. NH_4OH.
11.15 P. M. : 100 c.c. neutralized by 8.1 c.c. NH_4OH.
10.30 A. M. : 100 c.c. neutralized by 1.3 c.c. NH_4OH.

All determinations were made in triplicate, with varying volumes and the figures obtained agreed closely. These relative results show that at least 50 per cent of the total free acid was neutralized during the first three hours of contact with the bones.

[2] The quantity of dilute acid used for decalcification was about a litre for each portion of femur 6–8 inches in length; only the diaphysis was employed. When placed for a few hours in hydrochloric acid as dilute as 0.05 per cent, very thin, delicate shavings, so light that they float in water and dilute alcohol, may be obtained. Treatment with 0.5 per cent hydrochloric acid permits much more rapid decalcification, however, and makes the scraping process much easier. One half per cent hydrochloric acid was used in most of the experiments described in the second section, p. 402.

of superficial connective tissue elements belonging to the periosteum, which, perhaps, had not been completely removed in the preliminary treatment. The scraping process was continued until only a very thin, translucent layer inclosed the marrow cavity. While the shavings accumulated they were kept in 0.2 per cent hydrochloric acid for thorough decalcification, and for such gelatinization of collagenous elements as might be helpful to disintegration of the tissue and more complete liberation of "cement substance" during subsequent extraction. This treatment also prevented putrefactive changes.[1] At the end of two weeks two scrapings a day of two dozen sections of ox femur a little more than half a foot in length gave 1700 grams of moist ossein. The surplus moisture had been eliminated by cumulative pressure in a meat press.

The shavings were next run through a meat-chopper,[2] and then placed in running water until they were washed free from chloride. Finally the bulky ossein hash was transferred to several stoppered bottles and repeatedly shaken with half-saturated lime water in the proportion of from 2 to 5 c.c. of extractive fluid for every gram of the moist hash. Within ten minutes after the lime water treatment began, the extractive fluid became very frothy on shaking, and with excess of dilute acid a flocculent precipitate was obtained in a small portion. The extraction was continued for forty-eight hours, by the end of which time, it was subsequently found, almost all of the soluble substance had been removed. The filtered extract was then treated with 0.2 per cent hydrochloric acid.[3] The first addition produced heavy turbidity, and, after neutralization, a bulky flocculent precipitate separated at once in moderate excess of 0.2 per cent hydrochloric acid and fell rapidly to the bottom under a water-clear fluid.[4]

From this point the usual method for the purification of mucin was

[1] Subsequent experiments indicated that this acid treatment of the shavings, favoring gelatinization, is not particularly advantageous, perhaps is undesirable. Dilute alcohol (10 per cent) has been found to serve very well for preservative purposes during this preliminary period. See methods, p. 404 *et seq.*

[2] This can be done quite easily before the acid is washed out of the shavings, but is very difficult thereafter.

[3] Preferred to acetic acid as precipitant, because of its greater solvent action on non-glucoproteid material and because former experience has shown that connective tissue mucin is more easily thrown down with it.

[4] The precipitate closely resembled, in appearance and behavior, tendon mucin and chondromucoid.

pursued. The precipitate was several times washed, by decantation, in water made slightly acid with hydrochloric acid, then freed from acid by washing in water, filtered off, later dissolved in half-saturated lime water, reprecipitated with 0.2 per cent hydrochloric acid, repeatedly washed in acidified water, in water, and in alcohol, and lastly treated with boiling anhydrous alcohol-ether (50 per cent) as long as anything dissolved out. The alcohol was washed out with anhydrous ether. The purified substance dried quickly in the air to a very light, white, or faintly cream-colored powder devoid of hygroscopic qualities. Seventeen hundred grams of moist femur ossein yielded a trifle more than 7 grams of the substance; 875 grams of rib shavings gave 3.5 grams. In each case the amount of prepared substance was equal to approximately 0.4 per cent of the moist ossein.[1]

The acid filtrate from the substance thus prepared contains gelatin and a body closely related to, if not identical with, the separated mucoid. Possibly chondroitin sulphuric acid and gelatin combinations, such as Schmiedeberg[2] recognized, are in solution. The author is not sure that nucleoproteid is not contained in it. These matters are under investigation.

DISCUSSION OF MODIFYING FACTORS.

It will be seen from the analytic results given on page 402 that the substance which has been isolated by the method just described is typical glucoproteid. In considering its preparation by this method the author would not ignore the possibility that chondroitin sulphuric acid has combined with some of the gelatin, resulting from the action of the acid on the collagen, to form an artificial glucoproteid. It is well known that such combination of these substances may occur after prolonged contact at body temperature or more quickly in the presence of free acid, and it might be assumed that such syntheses took place in these experiments. Mörner found that chondroitin sulphuric acid has strong affinity for gelatin, in acidified solution, and made use of this tendency to detect the

[1] Various minor improvements of the method of preparation suggested themselves as the work progressed. Notes of these are made in the second section, p. 404 *et seq.*

[2] SCHMIEDEBERG: Archiv für experimentelle Pathologie und Pharmakologie, 1891, xxviii, p. 355.

ethereal compound.[1] Schmiedeberg[2] has given the names "peptochondrin" and glutinchondrin" to the insoluble intermediate combinations of gelatin pepton and chondroitin sulphuric acid, and "chondralbumin" or "chondralbuminoid" to the soluble products, formed in his process of isolating chondroitin sulphuric acid from cartilage. His experiments clearly indicate that various substances containing chondroitin sulphuric acid, similar to chondromucoid, are present in cartilage, probably all of them loose compounds of the acid with simple proteid. Mörner[3] has shown that chondroitin sulphuric acid may combine with simple proteid in the urine, which compound, on acidification, separates as an insoluble substance having most of the qualities of uromucoid. Krawkow[4] has also called attention to the fact that various combinations of chondroitin sulphuric acid may be induced with different proteids.

It has frequently been said that bone contains a trace of chondroitin sulphuric acid, but if any is present as such in osseous tissue, or as a simple alkali salt, it would seem that the author's preliminary treatment in these experiments should have entirely extracted it from the ossein, unless, perhaps, the hydrochloric acid, used to remove inorganic matter, fixed it *in situ* by quickly furnishing it with the requisite amount of gelatin before its solution from the decalcifying tissue. Mörner,[5] it will be recalled, used essentially this same acid treatment to gelatinize the collagen of cartilage in order to extract chondromucoid more completely and easily. After preliminary treatment with distilled water he digested the cartilage shavings in 0.1–0.2 per cent hydrochloric acid at 40° C. to transform insoluble collagen into soluble gelatin, thus disintegrating the tissue somewhat and favoring subsequent extraction of the glucoproteid from the residue with 0.05–0.1 per cent potassium hydroxide. Although it would be expected that this preliminary treatment with water should

[1] C. Th. Mörner: *Loc. cit.* The precipitate of gelatin and chondroitin sulphuric acid is readily soluble in excess of mineral acids. Salts interfere with precipitation of the compound by 0.2 per cent hydrochloric acid. Chondroitin sulphuric acid itself interferes to a certain extent with precipitation of chondromucoid by dilute acid at room temperature. See also, Zeitschrift für physiologische Chemie, 1894, xx, p. 357, and K. A. H. Mörner, cited in note below.

[2] Schmiedeberg: *Loc. cit.*

[3] K. A. H. Mörner: Skandinavisches Archiv für Physiologie, 1895, vi, p 332.

[4] Krawkow: Archiv für experimentelle Pathologie and Pharmakologie, 1897, xl, p. 195.

[5] C. Th. Mörner: *Loc. cit.*

suffice to dissolve out all of the preformed or loosely combined chondroitin sulphuric acid, it is possible that some of it may have remained in the cartilage in Mörner's experiments, just as some might have remained in the decalcified tissue in the present experiments. Mörner has ignored the matter entirely, and no one else has called attention to such possibility. The question raised in this connection is now being studied. The author inclines to the belief that artificial glucoproteid was not formed in the ossein in the manner just discussed.

It should not be forgotten, of course, in any consideration of this matter, that no one has ever shown definitely the existence of preformed, free chondroitin sulphuric acid in normal bones. Mörner's[1] first researches on the distribution of chondroitin sulphuric acid in the bones of the ox did not disclose its presence. Unlike Schmiedeberg,[2] however, he was able to prepare it from some pathological human cartilaginous and osseous structures — in six cases of enchondroma, in one of chondroma osteoides mucosum tibiae and one of exostosis cartilaginea humeri. Mörner's method of detecting chondroitin sulphuric acid in these investigations, consisting, as it did in part, of treatment with 2 per cent potassium hydroxide, makes it uncertain whether this complex ethereal sulphuric acid existed as such in the bones he analyzed or whether it was derived from preexistent glucoproteid in the extraction process.[3] The present writer thinks the latter view more probable.

Later, Mörner's[4] studies of the content of sulphuric acid in the ash of the bones of the ox, as well as in the acid extract obtained by treatment of bones from the same animal with boiling hydrochloric acid (25 per cent), led to the deduction that the constant trace of SO_3 found, 0.01–0.04 per cent, came from a very slight quantity of chondroitin sulphuric acid, and Mörner assumed that these indirect methods gave positive proof of the presence of this substance in bone, contrary to the former negative results, because of the "greater delicacy" they possessed over his original direct estimations. His methods of detection do not warrant the belief, however, that the SO_3

[1] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1895, xx, p. 357.

[2] Schmiedeberg: *Loc. cit.*

[3] Levene has separated a substance similar to chondroitin sulphuric acid from tendon mucin and other mucoids. Cleavage was accomplished by essentially the same treatment — with 2 per cent sodium hydroxide: Zeitschrift für physiologische Chemie, 1901, xxxi, p. 395. See also Schmiedeberg, *loc. cit.*, for similar facts.

[4] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 311.

came directly from preformed chondroitin sulphuric acid or an alkali salt. It might have come indirectly from glucoproteid, which, if present, would have been decomposed into simple proteid and SO_3 combinations during the treatment in each of the processes used.[1] Bielfeld[2] recently found as much as 0.076 per cent of SO_3 in the ash of fœtal bones and attributed this increase over Mörner's figures to a greater amount of chondroitin sulphuric acid in the embryonic tissue. It is quite as reasonable to assume, however, that the SO_3 detected by Bielfeld was originally a part of chondroitin sulphuric acid in constituent glucoproteid. Krawkow[3] also states that he found chondroitin sulphuric acid in the diaphysis of the femur of the horse, sheep, and ox. He decalcified with hydrochloric acid; he does not state the strength of the acid employed, but it may have been sufficient to decompose mucoid. Subsequently the prepared ossein was digested in artificial gastric juice (with probable formation of "peptochondrin," etc.), and chondroitin sulphuric acid was extracted from the undigested residue, after treatment with potassium hydroxide (amount and strength not stated), in continuation of Schmiedeberg's process. The methods Krawkow employed make it probable that the ethereal compound was derived from antecedent complex material, and his results prove nothing regarding preformed chondroitin sulphuric acid, or the presence in bone of a simple salt of the same.

PROPERTIES OF OSSEOMUCOID.

The substance prepared by the method previously outlined has the general qualities of the glucoproteids, and for the sake of convenient reference the author proposes for it the name osseomucoid, although he believes that it is quite as nearly related to the mucins of tendon and ligament[4] as is chondromucoid of cartilage.[5]

[1] See VANDEGRIFT and GIES: This journal, 1901, v, p. 287, for similar facts connected with SO_3 in the ash of ligament and for related points. Krawkow has separated chondroitin sulphuric acid by destructive method from ligamentum nuchæ as well as from bone.

[2] BIELFELD: Zeitschrift für physiologische Chemie, 1898, xxv, p. 350.

[3] KRAWKOW: *Loc. cit.*

[4] RICHARDS and GIES: Proceedings of the American Physiological Society; This journal, 1901, v, p. xi. Also, CUTTER and GIES: *Loc. cit.*

[5] Long after the completion of the experiments described under this head, and shortly before this paper was sent to the editor, the author received Cohnheim's Chemie der Eiweisskörper (1900) and was surprised to find, on page 285, the following: "The ground-work of bone, apart from a very slight quantity of mucoid

Osseomucoid dissolves readily in 0.05 per cent sodium carbonate and in 5 per cent sodium chloride, from which solutions it may be precipitated with mineral or organic acids. It appears to dissolve only slightly in cold 0.2 per cent hydrochloric acid. The moist substance is acid to litmus, lacmoid, and congo red. When the pure product, which had been precipitated with hydrochloric acid, was thoroughly decomposed in dilute nitric acid no chlorine reaction could be obtained in the fluid with silver nitrate. Like tendon and liga-

(mucin) and chondroitin sulphuric acid *which perhaps are not contained in true bone*, consists of collagen, etc." Cohnheim bases this statement regarding possible presence of mucoid on the authority of some observations of Morochowetz (Verhandl. d. Heidelberger naturh.-med. Vereins, N. F., i, p. 480, 1876), whose opinion in this particular connection seems to have received no attention at the time (the text-books of his day do not refer to it), and appears to have been entirely overlooked until Cohnheim brought it to light again (see historical review, p. 387). The only other reference to Morochowetz's work the author has had access to, in the absence of the original paper, is the abstract in the Jahresbericht über die Fortschritte der Thierchemie, 1877, p. 37, where, it may be seen, the article was entitled: "Zur Histochemie des Bindegewebes." Unfortunately, the abstract fails to mention bone among the tissues examined, which suggests, of course, that Morochowetz's result or statement in connection with it was a minor one. From the title of the paper it may be inferred that if any work was done on bone it was purely histochemical in nature and that no mucoid substance was really separated or accurately identified. Besides — and this is a point of considerable significance in this connection — the body which Morochowetz identified in the various other tissues under examination and which he called mucin, did not, he says, contain sulphur, a statement clearly indicating inaccurate chemical observation, since all of the connective tissue mucins contain a relatively large proportion of sulphur. From Cohnheim's statement it may also be judged that the mucoid to which Morochowetz referred was not definitely ascertained to be a part of true osseous tissue. On discovering the statement in Cohnheim's book, the author wrote at once to his colleague, Dr. H. C. Jackson, lately in Professor Hofmeister's laboratory, for detailed information as to the contents of Morochowetz's paper. Dr. Jackson consulted the original in the Strassburg library and, thanks to his kindness, the author is able to say that Morochowetz claimed to have obtained mucin (a sulphur-free glucoproteid!) from several forms of connective tissue, such as cornea and cartilage. The only form of bone studied was embryonic in structure and consequently contained much pure cartilage. Morochowetz states he obtained the same substance from fœtal bone that he had previously identified in various forms of cartilage. His deductions are to be referred rather to cartilage, therefore, than to true bone.

Since the above was given to the printer the author received, through the courtesy of Dr. Leon Asher, of Bern University, a reprint of Morochowetz's paper in the Heidelberg Verhandlungen. A study of the same confirms all that has been said here regarding it.

ment mucins, and chondromucoid, it dissolves in dilute alkali, and when sufficient substance is suspended in the liquid, neutralization of the latter results with formation of an alkali salt of the proteid, which is soluble in neutral fluid. Osseomucoid gives the biuret, Millon's, and the xanthoproteic reactions very distinctly. Neutral solutions of its salts are not coagulated on boiling. It gives only a slight sulphide reaction with lead acetate after decomposition in hot potassium hydroxide. The fluid containing the products of its decomposition by boiling 2 per cent hydrochloric acid, however, gives a heavy precipitate of barium sulphate with barium chloride in the presence of free hydrochloric acid, and strong reduction of Fehling's and Nylander's solutions may be obtained after neutralization. This carbohydrate substance yields osazone crystals with phenylhydrazin. Osseomucoid is partly digested in "pepsin-hydrochloric acid;" the anti-albumid-like residue probably contains substance similar to peptochondrin. On hydration in boiling mineral acid, anti-albumid, albuminate, proteose and pepton are formed and have been identified.

The original preparations, one from the rib, the other from the femur, of the ox, were partially analyzed, with the results shown in the table on page 402.[1]

The discovery of a mucoid constituent of bone naturally suggests numerous lines of investigation, some of which have already been indicated. In what quantity, for example, does osseomucoid exist in bone at various stages of development? Is it peculiar to some bones or is it found in all? How has it affected previous analyses of bone gelatin, of bone ash, etc.? What is its biological significance; its relation, if any, to pathological formations, its exact place in the glucoproteid classification; its inner make-up, composition reactions, etc. These and other related problems are under investigation and the author hopes to present detailed results of these studies in the near future. The following sections, on composition and heat of combustion, give complete results of some of the work in this general plan.

[1] The analyses were incomplete, only because the bulk of each preparation was used for the qualitative determinations which were necessary for ascertaining the general properties of the substance. The methods employed were the same as those outlined on p. 403 of the following section. Customary quantities were used. Sulphur was not determined in the ash because bone contains merely traces of sulphate and the reagents were free from it. Probably only that derived, on oxidation, from the proteid itself, would be found in the ash. Complete analytic results are given in the succeeding section.

In concluding this section, the author wishes to acknowledge his indebtedness to Mr. Christian Seifert, assistant in this laboratory, for much valuable help. Mr. Seifert carefully prepared, under the author's supervision, all of the bone shavings used in these experiments and cheerfully accomplished that arduous task at the cost of considerable personal inconvenience.

PERCENTAGE COMPOSITION.

Preparation.	Nitrogen.	Total sulphur.	Sulphur combined as SO_3.	Total phosphorus.	Ash phosphorus.	Ash.
A. Rib.	12.78	1.68	0.98	0.086	0.051	2.28
	12.99	1.75	0.91	0.031	0.039	2.19
	12.80					
	12.91					
B. Femur	13.38	1.89	1.04	0.108	0.057	2.62
	13.41	1.87	1.11	0.054	0.061	2.57
	13.45					
Calculated for ash-free substance.[1]						
A.	13.17	1.76	0.97	0.013		
B.	13.77	1.93	1.11	0.022		

II. COMPOSITION OF OSSEOMUCOID.[2]

The results of the preliminary analyses seemed to establish beyond doubt the general glucoproteid nature of osseomucoid. Complete elementary analysis was necessary, however, to determine definitely its chemical relationships. We have made such analyses of a number of additional products from the femur of the ox, which were prepared

[1] Reference to phosphorus content, and other deductions as to chemical relationship, are deferred to the succeeding section, where more complete analyses are given. See p. 412.

[2] HAWK and GIES: Proceedings of the American Physiological Society (Baltimore meeting, December, 1900). This journal, 1901, v, p. xv. Previous reports noted on p. 387.

and purified, with several variations, as will be indicated, by the method already given.[1] The results obtained in this work harmonize, it will be seen, with the original deductions.

METHODS OF ANALYSIS.

Carbon and hydrogen. — Estimations were made, with all due precautions, by the method of oxidation in properly arranged combustion tubes, the gaseous products formed in the process passing through a layer of granulated copper oxide and over a reduced copper spiral. The absorbing apparatus consisted of three U-tubes of suitable size, containing concentrated sulphuric acid in the first, for the absorption of water, soda lime in the second and soda lime, with pumice stone moistened by sulphuric acid, in the third, for the absorption of carbon dioxide.[2] The soda lime was prepared as recommended by Benedict.[3] The tubes of the absorbing apparatus were wiped with cloth, in all cases, before weighing, and finally weighed upon a counterpoised balance until constant figures were obtained.[4]

Nitrogen. — Nitrogen was determined by the Kjeldahl process. Digestion of the substance in concentrated sulphuric acid was completed with small quantities of metallic mercury. Before distillation with excess of caustic soda, the mercury was precipitated with potassium sulphide. In the titrations, congo red was used as the indicator.

Total sulphur a d phosphorus. — These elements were determined by the well known fusion methods. Fusion was made in silver crucibles (over alcohol flames in the sulphur determinations), with solid potassium hydroxide and potassium nitrate, each free from phosphorus and sulphur.[5]

Sulphur combined as SO_3. — Sulphur in the form of ethereal sulphuric acid was determined as follows: The substance was digested with about 175 c.c. of 2 per cent hydrochloric acid over an alcohol flame for six hours in a flask connected with a reflux condenser. At the end of the boiling process, when cleavage was complete, the

[1] See p. 393.

[2] Benedict: Elementary organic analysis, 1900, p. 34.

[3] Benedict: Journal of the American Chemical Society, 1899, xxi, p. 393.

[4] An important precaution. Considerable variation in the results may occur when it is not observed.

[5] When traces of these elements were present in the reagents, their quantities were carefully determined and corrections made accordingly.

acidity of the fluid was reduced somewhat with pure ammonium hydroxide, although the mixture was left distinctly acid. It was then filtered for the separation of antialbumid-like substance which had formed in small proportion during the process. The sulphuric acid in the hot filtrate and washings finally was precipitated with barium chloride, and the figures for sulphur obtained from the barium sulphate in the usual manner.[1]

Ash. — Inorganic matter was estimated by direct incineration of the substance in a platinum crucible over a very low flame. Phosphorus of the ash was determined in nitric acid solution of the same by the customary method involving the use of "molybdic solution" and "magnesia mixture."[2]

RECORDS OF ANALYSIS.[3]

Preparation No. 1. — Bones in 0.3 per cent HCl. 2,700 grams moist shavings accumulated in 0.2 per cent HCl. Before extraction in lime water, acid was removed by washing in large volumes of water. When decanted fluid no longer gave acid reaction to litmus, ossein hash was extracted in half-saturated lime water, 4 c.c. of dilute alkali per gram of substance, for forty-eight hours. End of that time, extract neutral; gave only slight precipitate on acidification with 0.2 per cent HCl. Acid had not been completely washed out by decantation method. Hash placed in half-saturated lime water again; same quantity for same time. Second extract gave excellent precipitate on acidification with 0.2 per cent HCl. Slight precipitate of first extract discarded, only second purified. Dissolved in half-saturated lime water, filtrate opalescent. Reprecipitated once with 0.2 per cent HCl. Washed in water, alcohol, ether, etc. Purified product snow-white, very light, amorphous powder. 6.5 grams. Dried to constant weight at 100-110° C. and analyzed with following results:

Carbon and Hydrogen.[4] 0.1520 gram substance gave 0.2667 gram CO_2 = 47.85 per cent C, and 0.0952 gram H_2O = 7.01 per cent H; 0.1728 gram substance gave 0.3046 gram CO_2 = 48.08 per cent C, and 0.1078 gram H_2O = 6.98 per cent H.

[1] Great care was taken to prevent introduction of sulphate during the method of preparation of the osseomucoid analyzed. The reagents used were entirely free from SO_3.

[2] Sulphur of the ash was not determined. See note, p. 401.

[3] Very brief reference to the more important details of preparation precedes the analytic data of each particular sample of osseomucoid. The method given on p. 393 is followed in a general way for each preparation.

[4] Osseomucoid is so light and bulky that larger quantities of substance could hardly be used conveniently in these determinations. Special care was exercised, herefore, in all the analyses.

Nitrogen. 0.2606 gram substance gave 0.0369 gram N = 14.15 per cent N; 0.2557 gram substance gave 0.0361 gram N = 14.11 per cent N; 0.2520 gram substance gave 0.0354 gram N = 14.06 per cent N.

Total Sulphur. 0.2518 gram substance gave 0.0204 gram $BaSO_4$ = 1.12 per cent (?) S; 0.2530 gram substance gave 0.0249 gram $BaSO_4$ = 1.36 per cent S; 0.2510 gram substance gave 0.0252 gram $BaSO_4$ = 1.38 per cent S.

Sulphur combined as SO_3. 0.2390 gram substance, after boiling in HCl, gave 0.0103 gram $BaSO_4$ = 0.59 per cent S; 0.2418 gram substance, after boiling in HCl, gave 0.0085 gram $BaSO_4$ = 0.49 per cent S.

Ash. 0.3134 gram substance gave 0.0070 gram Ash = 2.24 per cent Ash; 0.2560 gram substance gave 0.0054 gram Ash = 2.11 per cent Ash; 0.2572 gram substance gave 0.0064 gram Ash = 2.49 per cent Ash.

Total Phosphorus. 0.2509 gram substance gave 0.0009 gram $Mg_2P_2O_7$ = 0.099 per cent P; 0.2516 gram substance gave 0.0007 gram $Mg_2P_2O_7$ = 0.078 per cent P.

Ash Phosphorus. 0.8266 gram substance left 0.0187 per cent Ash, which gave 0.0008 gram $Mg_2P_2O_7$ = 0.029 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	48.97	49.20					...	49.08
H	7.17	7.14						7.16
N			14.48	14.44	14.39			14.44
S						1.39	1.41	1.40
O								27.92

Preparation No. 2. — Preliminary treatment same as in Prep. No. 1, except that bones were decalcified in 0.5 per cent HCl. 1,900 grams moist shavings. Profiting by previous experience, however, acid was washed out in running water. Extraction made in 10 c.c. half-saturated lime water for each gram of ossein; continued twenty hours. 2.5 per cent acetic acid used to precipitate. Substance separated in large flocks and settled out more slowly than when thrown down by dilute HCl. Dissolved in half-saturated lime water. Filtrate slightly turbid or opalescent in spite of repeated filtration. Reprecipitated once with 2.5 per cent acetic acid in moderate excess. Washed in water, alcohol, etc. Partly gummy on drying. 5.7 grams dried at 100-110° C. and analyzed, with appended results:

Carbon and Hydrogen. 0.1273 gram substance gave 0.2216 gram CO_2 = 47.48 per cent C, and 0.0815 gram H_2O = 7.16 per cent H; 0.1306 gram substance gave 0.2276 gram CO_2 = 47.53 per cent C, and 0.0777

[1] Reference to phosphorus content is made on p. 412.

gram H_2O = 6.66 per cent (?) H; 0.1280 gram substance gave 0.2242 gram CO_2 = 47.77 per cent C, and 0.0834 gram H_2O = 7.29 per cent H.

Nitrogen. 0.2522 gram substance gave 0.0348 gram N = 13.79 per cent N; 0.2188 gram substance gave 0.0305 gram N = 13.94 per cent N; 0.2484 gram substance gave 0.0349 gram N = 14.02 per cent N.

Total Sulphur. 0.2037 gram substance gave 0.0210 gram $BaSO_4$ = 1.42 per cent S; 0.2035 gram substance gave 0.0202 gram $BaSO_4$ = 1.37 per cent S.

Sulphur combined as SO_3. 0.2021 gram substance, after boiling in HCl, gave 0.0089 gram $BaSO_4$ = 0.61 per cent S; 0.2035 gram substance, after boiling in HCl, gave 0.0105 gram $BaSO_4$ = 0.71 per cent S.

Ash. 0.2556 gram substance gave 0.0066 gram Ash = 2.58 per cent Ash; 0.2528 gram substance gave 0.0064 gram Ash = 2.53 per cent Ash.

Total Phosphorus. 0.2012 gram substance gave 0.0006 gram $Mg_2P_2O_7$ = 0.083 per cent P; 0.3127 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.045 per cent P.

Ash Phosphorus. 0.5084 gram substance left 0.0130 gram Ash, which gave 0.0007 gram $Mg_2P_2O_7$ = 0.038 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

									Average.
C	48.72	48.77	49.01						48.83
H	7.35		7.48						7.42
N				14.15	14.30	14.38			14.27
S							1.46	1.41	1.43
O									28.05

Preparation No. 3. — Preliminary treatment same as for Prep. No. 2. 2,900 grams moist shavings. Two extractions made; first for twenty hours, second for seventy-two hours. Osseomucoid precipitated by 0.2 per cent HCl. Much less substance precipitated from second extract than from first. Combined and dissolved in 0.05 per cent KOH. Filtrate slightly opalescent. Thrice reprecipitated by 0.2 per cent HCl.[2] Then washed once in 0.1 per cent HCl, lastly in H_2O, etc. 11.2 grams light cream colored powder. Dried, etc., with following analytic results:

Carbon and Hydrogen. 0.1106 gram substance gave 0.1858 gram CO_2 = 45.82 per cent C, and 0.0681 gram H_2O = 6.89 per cent H; 0.1143 gram substance gave 0.1946 gram CO_2 = 46.43 per cent (?) C, and

[1] It will be observed that the composition of the product precipitated by acetic acid (Prep. No. 2) is essentially the same as that prepared with 0.2 per cent hydrochloric acid (Prep. No. 1).

[2] Extra reprecipitation seems to have resulted in lowering of the percentage of carbon and nitrogen, and raising that of sulphur and oxygen. See p. 407.

0.0698 gram H_2O = 6.83 per cent H; 0.0970 gram substance gave 0.1627 gram CO_2 = 45.75 per cent C, and 0.0620 gram H_2O = 7.15 per cent H; 0.1075 gram substance gave 0.1810 gram CO_2 = 45.92 per cent C, and 0.0680 gram H_2O = 7.08 per cent H.

Nitrogen. 0.2790 gram substance gave 0.0366 gram N = 13.13 per cent N; 0.3281 gram substance gave 0.0433 gram N = 13.20 per cent N; 0.2651 gram substance gave 0.0348 gram N = 13.12 per cent N.

Total Sulphur. 0.2526 gram substance gave 0.0336 gram $BaSO_4$ = 1.83 per cent S; 0.2516 gram substance gave 0.0332 gram $BaSO_4$ = 1.82 per cent S.

Sulphur Combined as SO_3. 0.2434 gram substance, after boiling in HCl, gave 0.0183 gram $BaSO_4$ = 1.03 per cent S; 0.2438 gram substance, after boiling in HCl, gave 0.0181 gram $BaSO_4$ = 1.02 per cent S.

Ash. 0.2602 gram substance gave 0.0039 gram Ash = 1.50 per cent Ash; 0.2589 gram substance gave 0.0040 gram Ash = 1.54 per cent Ash.

Total Phosphorus. 0.2504 gram substance gave 0.0009 gram $Mg_2P_2O_7$ = 0.100 per cent P; 0.2506 gram substance gave 0.0004 gram $Mg_2P_2O_7$ = 0.045 per cent P; 0.2874 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.048 per cent P.

Ash Phosphorus. 0.5191 gram substance left 0.0079 gram Ash, which gave 0.0003 gram $Mg_2P_2O_7$ = 0.016 per cent P.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.

										Average.
C	46.53		46.46	46.63						46.54
H	7.00	6.94	7.26	7.19						7.10
N					13.33	13.40	13.32			13.35
S								1.86	1.85	1.85
O										31.16

Preparation No. 4. — Same preliminaries as for Prep. No. 2. 3,950 grams moist shavings. Extraction in 10 c.c. half-saturated lime water for each gram of ossein; continued seventy-two hours. Osseomucoid precipitated with 0.2 per cent HCl. Dissolved in slight excess of 0.05 per cent NaOH and reprecipitated five times; each solution filtered. Filtrate at first turbid or opalescent as each time heretofore. After the pores of the filter paper became clogged, however, the filtrate was collected more slowly, but came through as clear as water, though yellowish in color.[1] About three-

[1] Possibly the observed differences in analytic results between this and the previous preparations were due to the presence of bone corpuscles, etc., in the latter, which had not been completely removed in the process of filtration. See also foot-note, p. 406.

fourths of final solution obtained water clear; turbid portion discarded. After fifth reprecipitation substance was thoroughly stirred up in 8 litres of 0.2 per cent HCl. There was no particular diminution in quantity, although the flocks seemed to shrink somewhat and become heavier and more granular. Acid washed out with H_2O. Final purification same as heretofore. In spite of losses, 7.7 grams osseomucoid obtained; very light, cream colored. Analyzed in the usual way, the appended results were obtained:

Carbon and Hydrogen. 0.1124 gram substance gave 0.1906 gram CO_2 = 46.25 per cent C, and 0.0669 gram H_2O = 6.66 per cent H; 0.1311 gram substance gave 0.2216 gram CO_2 = 46.14 per cent C, and 0.0797 gram H_2O = 6.81 per cent H.

Nitrogen. 0.2670 gram substance gave 0.0320 gram N = 11.97 per cent N; 0.2810 gram substance gave 0.0339 gram N = 12.06 per cent N.

Total Sulphur. 0.2526 gram substance gave 0.0406 gram $BaSO_4$ = 2.21 per cent S; 0.2534 gram substance gave 0.0373 gram $BaSO_4$ = 2.03 per cent S; 0.3032 gram substance gave 0.0406 gram $BaSO_4$ = 1.84 per cent (?) S; 0.3290 gram substance gave 0.0503 gram $BaSO_4$ = 2.10 per cent S.

Sulphur Combined as SO_3. 0.3227 gram substance, after boiling in HCl, gave 0.0259 gram $BaSO_4$ = 1.10 per cent S; 0.3237 gram substance, after boiling in HCl, gave 0.0251 gram $BaSO_4$ = 1.04 per cent S.

Ash. 0.2662 gram substance gave 0.0012 gram Ash = 0.45 per cent Ash; 0.2656 gram substance gave 0.0012 gram Ash = 0.45 per cent Ash.

Total Phosphorus. 0.3022 gram substance gave 0.0004 gram $Mg_2P_2O_7$ = 0.044 per cent P; 0.3028 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.018 per cent P.

Ash Phosphorus. 0.5318 gram substance left 0.0024 gram Ash, which gave 0.0003 gram $Mg_2P_2O_7$ = 0.016 per cent P.

Percentage Composition of the Ash-free Substance.

								Average.
C	46.46	46.35						46.40
H	6.69	6.84						6.77
N			12.02	12.11				12.06
S					2.22	2.04	2.11	2.12
O								32.65

Preparation No. 5. Bones decalcified in 0.5 per cent HCl. In sixteen days 4,410 grams moist shavings obtained. Shavings each day were placed in 0.1 per cent HCl; on the following day, and thereafter until used, in 25 per cent alcohol. Latter was acid from acid in shavings. Acid washed out with water by decantation until pieces of the ossein hash no longer reacted acid to litmus. 6 c.c. half-saturated lime water used to extract, for each gram of ossein. After two hours, extract was nearly neutral; showing that acid in interior of pieces

had not been completely washed out.[1] Sufficient 10 per cent KOH was then added, drop by drop with thorough shaking, to make approximately 0.05 per cent KOH in the fluid. After twelve hours the alkalinity had again perceptibly diminished; 2 c.c. half-saturated lime water for each gram of ossein finally added. Extractive period, from beginning, was fifty-two hours. Extract in the end very frothy. Was diluted with equal volume of water, and osseomucoid precipitated from diluted solution with 0.2 per cent HCl. Reaction was made only very slightly acid; precipitation purposely left incomplete, the turbid portion yielding small amount of flocculent precipitate on further acidification. This was discarded. Main precipitate dissolved in half-saturated lime water and reprecipitated eight times. Just before final precipitation with 0.2 per cent HCl, the filtrate, after passing through the same filter paper repeatedly, was obtained as clear as water. In the end poured into 0.2 per cent HCl drop by drop, with instantaneous precipitation. Substance finally washed in sixteen litres 0.2 per cent HCl and twenty-four litres 0.1 per cent HCl, with thorough stirring; eventually in water, alcohol, etc. During the washing in water, some of the product persisted in floating, as had been the case in all previous preparations. In this particular case the floating portion was finally skimmed off and discarded. 17.8 grams of cream colored fluffy powder were obtained. Dried and analyzed:

Carbon and Hydrogen. 0.1247 gram substance gave 0.2180 gram CO_2 = 47.68 per cent C, and 0.0718 gram H_2O = 6.44 per cent H; 0.1492 gram substance gave 0.2615 gram CO_2 = 47.80 per cent C, and 0.0877 gram H_2O = 6.58 per cent H; 0.1615 gram substance gave 0.2809 gram CO_2 = 47.44 per cent C, and 0.0938 gram H_2O = 6.50 per cent H.

Nitrogen. 0.3026 gram substance gave 0.0355 gram N = 11.75 per cent N; 0.3022 gram substance gave 0.0352 gram N = 11.64 per cent N.

Total Sulphur. 0.5674 gram substance gave 0.1020 gram $BaSO_4$ = 2.47 per cent S; 0.5306 gram substance gave 0.0969 gram $BaSO_4$ = 2.51 per cent S.

Sulphur combined as SO_3. 0.4026 gram substance, after boiling in HCl, gave 0.0452 gram $BaSO_4$ = 1.54 per cent S; 0.4018 gram substance, after boiling in HCl, gave 0.0572 gram $BaSO_4$ = 1.96 per cent (?) S; 0.3512 gram substance, after boiling in HCl, gave 0.0382 gram $BaSO_4$ = 1.50 per cent S.

Ash. 0.3542 gram substance gave 0.0010 Ash = 0.28 per cent Ash; 0.3518 gram substance gave 0.0009 gram Ash = 0.26 per cent Ash; 1.329 gram substance gave 0.0043 gram Ash = 0.32 per cent Ash.

Total Phosphorus. 0.6371 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.008 per cent P; 0.9381 gram substance gave 0.0007 gram $Mg_2P_2O_7$ = 0.021 per cent P.

Ash Phosphorus. 1.329 gram substance left 0.0043 gram Ash, which gave 0.0007 gram $Mg_2P_2O_7$ = 0.015 per cent P.

[1] See foot-note, p. 410.

PERCENTAGE COMPOSITION OF THE ASH-FREE SUBSTANCE.[1]

								Average.
C	47.82	47.94	47.58					47.78
H	6.46	6.60	6.52					6.53
N				11.78	11.67			11.72
S						2.48	2.52	2.50
O								31.47

Preparation No. 6. Bones in 0.5 per cent HCl eighteen days. 6,680 grams moist shavings obtained by end of that time. As they were made they were placed in 10 per cent alcohol, repeatedly renewed to remove acid during period of accumulation. Alcohol washed out later in water by decantation. Extraction in half-saturated lime water, 8 c.c. per gram of ossein. End of four hours, extract nearly neutral. 10 per cent KOH added as before to make 0.05 per cent KOH in extract. After eighteen hours, extract again nearly neutral. 10 per cent KOH added to make total of 0.1 per cent KOH. Alkalinity gradually decreased; due not only to combining power of osseomucoid but also, probably, to failure to completely wash out HCl.[2] Ossein in dilute alkali for ten days. Powdered thymol prevented putrefactive change. Extract finally obtained as perfectly clear filtrate. Diluted with four volumes water and this treated with equal volume 0.4 per cent HCl. Immediate precipitation in large flocks, which became smaller and more granular after thorough stirring, and quickly settled out. Precipitate dissolved in fifth-saturated baryta water and reprecipitated with 0.4 per cent HCl nine times. Tenth reprecipitation made by filtering the $3\frac{1}{2}$ litres of the baryta solution of substance into twenty litres of 0.2 per cent acid. Each drop solidified on contact and fell quickly to the bottom in globular form. Globules were broken up on stirring. Thoroughly washed in 0.3, 0.2 and 0.1 per cent HCl, later in water, etc., as usual. Final product very light, snow-white powder: 29.75 grams. Following results of analysis were obtained:

Carbon and Hydrogen. 0.1862 gram substance gave 0.3176 gram CO_2 = 46.52 per cent C, and 0.1114 gram H_2O = 6.65 per cent H; 0.1877 gram substance gave 0.3190 gram CO_2 = 46.36 per cent C, and 0.1128 gram H_2O = 6.68 per cent H; 0.1449 gram substance gave 0.2469 gram CO_2 = 46.47 per cent C, and 0.0906 gram H_2O = 7 per cent H; 0.1649 gram substance gave 0.2802 gram CO_2 = 46.34 per cent C, and 0.1013 gram H_2O = 6.87 per cent H.

[1] See foot-notes, pp. 406 and 407.

[2] It is evident that the decantation process must be repeated very frequently if all acid is to be washed out. Filtered running water serves best for this purpose.

Nitrogen. 0.3000 gram substance gave 0.0360 gram N = 12 per cent N; 0.3000 gram substance gave 0.0357 gram N = 11.90 per cent N; 0.3000 gram substance gave 0.0360 gram N = 12 per cent N.

Total Sulphur. 0.3887 gram substance gave 0.0734 gram $BaSO_4$ = 2.59 per cent S; 0.2761 gram substance gave 0.0502 gram $BaSO_4$ = 2.50 per cent S.

Sulphur combined as SO_3. 0.3045 gram substance, after boiling in HCl, gave 0.0344 gram $BaSO_4$ = 1.55 per cent S; 0.3355 gram substance, after boiling in HCl, gave 0.0379 gram $BaSO_4$ = 1.55 per cent S.

Ash. 0.2658 gram substance gave 0.0006 gram Ash = 0.23 per cent Ash; 0.2650 gram substance gave 0.0006 gram Ash = 0.23 per cent Ash; 1.3781 gram substance gave 0.0036 gram Ash = 0.26 per cent Ash.

Total Phosphorus. 0.6840 gram substance gave 0.0002 gram $Mg_2P_2O_7$ = 0.008 per cent P.

Ash Phosphorus. 1.3781 gram substance left 0.0036 gram Ash, which gave 0.0009 gram $Mg_2P_2O_7$ = 0.018 per cent P.

Percentage Composition of the Ash-free Substance.[1]

										Average.
C	46.63	46.47	46.58	46.45						46.53
H	6.67	6.69	7.01	6.89						6.81
N					12.03	11.93	12.03			11.99
S								2.60	2.51	2.55
O										32.12

Preparation No. 7. Fifty sections of femur decalcified in particularly dilute HCl — 0.05 per cent.[2] Scraped twice daily. Shavings, as they were collected, were placed directly into 3–5 litres of water, 12–24 hours, and then in 10 per cent alcohol until sufficient quantity accumulated. At end of three weeks 2,500 grams very thin, narrow, elastic shavings obtained. After hashing, the finely divided ossein was extracted in half-saturated lime water, 20 c.c. per gram of hash, for seventy-two hours. Alkalinity had perceptibly diminished by end of that time. Water clear filtrate obtained. With 0.2 per cent HCl in excess finely flocculent precipitate at once. Same purification process as for Prep. No. 6. Reprecipitated only five times. Final product very light, white powder; 5.2 grams. Analytic results as follows :

Carbon and Hydrogen. 0.2470 gram substance gave 0.4304 gram CO_2 = 47.51 per cent C, and 0.1487 gram H_2O = 6.69 per cent H; 0.1952

[1] See foot-notes, pp. 406 and 407.

[2] The analytic results of this preparation agree very well with those for preparations Nos. 5 and 6, and indicate that the acid used in decalcifying has had no particular influence on the products separated.

gram substance gave 0.3389 gram CO_2 = 47.35 per cent C, and 0.1158 gram H_2O = 6.59 per cent H.

Nitrogen. 0.1754 gram substance gave 0.0212 gram N = 12.05 per cent N; 0.2431 gram substance gave 0.0296 gram N = 12.18 per cent N.

Total Sulphur. 0.4482 gram substance gave 0.0783 gram $BaSO_4$ = 2.40 per cent S; 0.6320 gram substance gave 0.1158 gram $BaSO_4$ = 2.52 per cent S.

Sulphur combined as SO_3. 0.6171 gram substance, after boiling in HCl, gave 0.0678 gram $BaSO_4$ = 1.51 per cent S; 0.5009 gram substance, after boiling in HCl, gave 0.0501 gram $BaSO_4$ = 1.37 per cent S.

Ash. 0.7256 gram substance gave 0.0022 gram Ash = 0.30 per cent Ash; 0.2891 gram substance gave 0.0008 gram Ash = 0.28 per cent Ash.

Total Phosphorus. 0.5661 gram substance gave 0.0005 gram $Mg_2P_2O_7$ = 0.025 per cent P.

Ash Phosphorus. 1.0147 gram substance left 0.0030 gram Ash, which gave 0.0010 gram $Mg_2P_2O_7$ = 0.027 per cent P.

Percentage Composition of the Ash-free Substance.

							Average.
C	47.65	47.49					47.57
H	6.71	6.61					6.66
N			12.09	12.22			12.15
S					2.41	2.53	2.47
O							31.15

Summaries and Discussion of Analytic Results.

Content of phosphorus. — Before reviewing the general results of the analyses of the seven preparations we have carefully studied, special attention should be directed to the data on phosphorus content. The averages of our figures for percentage amount are here summarized: —

Substance.	Preliminary preparations.		Preparations completely analyzed.							Averages.
	Rib.	Femur.	1.	2.	3.	4.	5.	6.	7.	All.
Dry.	0.058	0.081	0.088	0.064	0.046	0.031	0.014	0.008	0.025	0.046
Ash.	0.045	0.059	0.029	0.038	0.016	0.016	0.015	0.018	0.027	0.029
Ash free.	0.013	0.022	0.049	0.026	0.030	0.015				0.017

It is very evident, from these results, that osseomucoid is a substance free from phosphorus. Not only are the above quantities entirely too small to have any particular significance, but all of them are within the ordinary variations in accuracy of the method of determination itself, and fluctuations may be due to unavoidable analytic error. Such traces as are indicated by the very painstaking determinations we have made undoubtedly are a part of the ash and not of the organic substance itself. The higher figures for the earlier preparations might be interpreted to mean phosphorized-proteid impurity. The differences are too slight, however, to warrant any such conclusion.[1]

Sulphur combined as SO_3. — We have not yet attempted to separate chondroitin sulphuric acid from osseomucoid, but the large proportion of combined SO_3 detected in, and separated from osseomucoid strongly indicates the presence of such a radicle in its molecule, particularly also because of the acid reaction of the proteid itself. The recent results obtained by Panzer,[2] on ovarial mucoid (paramucin), and Levene,[3] on various connective tissue and glandular glucoproteids, further suggest the probability that such an acid radicle will eventually be separated from osseomucoid. The percentage quantities of sulphur combined as SO_3 in all our preparations are here summarized, for ash-free substance, and the general averages contrasted with the amounts in chondromucoid and the mucins of ligament and tendon : — [4]

Preliminary preparations.		Preparations completely analyzed.							Averages.		Chondromucoid.	Tendon mucin.	Ligament mucin.
Rib.	Femur.	1.	2.	3.	4.	5.	6.	7.	All.	4–7.	Averages.		
0.97	1.11	0.55	0.68	1.05	1.08	1.53	1.55	1.44	1.11	1.40	1.76	1.43	1.07

[1] The tendon mucins analyzed by Dr. Gies, several years ago, contained 0.17 per cent P (average), which was also found to equal the percentage of phosphate in the ash. This observation has since been verified by Mr. Cutter, and identical results obtained for ligament mucin by Dr. Richards, in this laboratory. See also Krawkow's figures for percentage of amyloid : Krawkow, *loc. cit.*

[2] Panzer : *Loc. cit.*

[3] Levene : *Loc. cit.*

[4] C. Th. Mörner, Cutter and Gies, Richards and Gies : *Loc. cit.*

General Review. — The appended table summarizes the results for average percentage composition of osseomucoid (ash-free substance) and gives average composition of preparations 1–7; also of preparations 4–7, inclusive, the latter having been specially grouped together because of the greater attention given to their purification, by repeated reprecipitation, as has already been indicated: —

Elements.	Individual preparations.							Averages.	
	1.	2.	3.	4.	5.	6.	7.	1–7	4–7
C	49.08	48.83	46.54	46.40	47 78	46.53	47.57	47.53	47.07
H	7.16	7.42	7.10	6.77	6.53	6.81	6.66	6.92	6.69
N	14.44	14.27	13.35	12.06	11 72	11.99	12.15	12.85	11.98
S	1.40	1.43	1.85	2.12	2 50	2.55	2.47	2.05	2.41
O	27.92	28.05	31.16	32.65	31.47	32.12	31.15	30.65	31.85

The above results emphasize the glucoproteid character of osseomucoid, for, like practically all of these compound proteids, osseomucoid has a relatively low content of carbon and nitrogen, with a comparatively large proportion of sulphur and oxygen — due to the content of carbohydrate (probably polysaccharide) and sulphuric acid radicles; both rich in oxygen, the latter in sulphur.

Lack of particular uniformity in percentage composition, however, is evident on comparing the analytic results for the individual preparations. This want of analytic harmony cannot be due to nucleoproteid impurity, — our results for content of phosphorus show that conclusively,[1] — nor does it seem probable that admixture of other soluble proteid can be the cause, for bone contains too little such material to warrant that belief. We have already considered the possibility of chondroitin sulphuric acid combining with any gelatin made during the process of decalcifying, to form different products of varying solubilities, but, as has already been suggested, there is no reason to believe that bone contains sufficient chondroitin sulphuric acid to

[1] The content of phosphorus is too low for an assumption that either nucleoalbumin (0.4–0.8 per cent P) or phosphoglucoproteid (0.45 per cent P) was admixed. Comparatively large quantities of the substance contained the merest trace of iron. Undoubtedly this minute amount is to be recognized as inorganic impurity.

effect such a result.[1] We varied our method of preparation somewhat each time a new product was made for analysis, as may be seen in the records of analytic results, but, unless it be assumed that osseomucoid is very unstable, like submaxillary mucin, for example, and therefore easily influenced by the mild chemical treatment to which it was subjected, these changed conditions would not account for altered composition. We have seen, however, that osseomucoid behaves like tendon mucin and chondromucoid. We have every confidence in the accuracy of our methods of analysis and their manipulation.

Hammarsten,[2] it will be remembered, found that frequent precipitation of submaxillary mucin resulted in a lowering of the percentage of carbon and nitrogen of the purified product because of fractional elimination of nucleoalbumin. Our preparations 4–7 were given particular attention in this regard, with general results similar to those obtained by Hammarsten, and it may be that we have had to deal with unsuspected proteid impurity, which could only be, and perhaps was finally, eliminated by repeated reprecipitation. In the absence of direct evidence of such impurity, however, — and every condition seems to be against its occurrence, — we think our results justify the conclusion that the mucin substance of bone varies in composition just as the glucoproteid from other sources does, and that the figures in our analyses represent the make-up of several of these very closely related bodies. Such a conclusion not only accords with our analytic results but harmonizes also with the deductions drawn, under similar conditions for other tissues and products, by various observers.[3]

There appear to be many forms of glucoproteid. In all probability the acid and carbohydrate radicles of the mucoids have the power of uniting with various proteids in varying proportions to form different compounds, and while they can easily be arranged into general groups as we classify them to-day, in inner make-up they are doubtless multifarious. Such a conception of the chemical nature of the mucin substances would account for the wide variations that have been observed in the elementary composition not only of apparently the same substance, but also of very nearly related products from differ-

[1] See p. 396.

[2] Hammarsten: Zeitschrift für physiologische Chemie, 1888, xii, p 163.

[3] Chittenden and Gies: The journal of experimental medicine, 1896, i, p. 186. Also, Schmiedeberg, K. A. H. Mörner, Cutter and Gies, Krawkow, Richards and Gies: *Loc. cit.*

GENERAL SUMMARY. AVERAGE PERCENTAGE COMPOSITION.

	Glucoproteids.										Simple proteid.
Elements.	Osseomucoid.		Chondromucoid.[1]	Tendon mucin.[2]	Cornea mucoid.[3]	Umbilical mucoid.[4]	Submaxillary mucin.[5]	Ovarial mucoid.[6]	Amyloid.[7]	Serum mucoid.[8]	Serum albumin.[9]
	1–7.	4–7.									
C.	47.53	47.07	47.30	48.76	50.16	51.33	48.84	51.76	49.44	47.60	53.08
H	6.92	6.69	6.42	6.53	6.97	6 63	6.80	7.76	6.79	7.10	7.11
N	12.85	11.98	12.58	11.75	12.79	14.13	12.32	10.70	13.92	12.93	15.93
S	2.05	2.41	2.42	2.33	2.07	1.04	0.84	1.09	2.79	2.38	1.90
O	30.65	31.85	31.28	30.63	28.01	26.87	31.20	28.69	27.06	29.99	21.98

[1] C. Th. Mörner: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.
[2] Chittenden and Gies: *Loc. cit.*
[3] C. Th. Mörner: Zeitschrift für physiologische Chemie, 1893, xviii, p. 213.
[4] Jernström: Jahresbericht über die Fortschritte der Thier-Chemie, 1880, x, p. 34.
[5] Hammarsten: *Loc. cit.*
[6] Mitjukoff: Centralblatt für die medicinischen Wissenschaften, 1895, xxxiii, p. 737.
[7] Krawkow: *Loc. cit.*
[8] Zanetti: Jahresbericht über die Fortschritte der Thier-Chemie, 1897, xxvii, p. 31.
[9] Michel: *Ibid.*, 1895, xxv, p. 11.

ent tissues. Until we know more about the inner nature of simple proteid, and of such complex substances as chondroitin sulphuric acid which readily unite with proteid in the normal and pathological metabolic changes in the tissues, it will be difficult to reach, from analytic results, conclusions more definite regarding various glucoproteids than those we have been able to draw from our analyses of osseomucoid.

Compared results. — In the general summary, on page 416, of analytic figures for tissue mucoids, direct comparison may be made with the osseomucoid averages. The figures for crystallized serum albumin are also given for convenient comparison of the collated analytic data with similar results for simple proteid.

III. Heat of Combustion of Osseomucoid, Tendon Mucin and Chondromucoid.

HISTORICAL.

In any consideration of the metabolism of energy in the body, the combustion equivalents of the food and excreta are factors of fundamental importance. It is now generally agreed, we believe, by all who have given special attention to such studies, that careful determinations of the potential energy, as expressed in calories, of all the constituents of the tissues should be made, if various important phases of metabolism are to be more thoroughly comprehended.

Although the "fuel values" of numerous albuminous mixtures, and some proteid substances, taken from the animal body have been very carefully estimated, no attention appears to have been paid, in this connection, to the glucoproteids, members of which group of bodies constitute so large a proportion of the interfibrillar or intercellular substance of various forms of connective tissue. We considered it desirable, therefore, to determine the combustion equivalent of osseomucoid and also of related glucoproteid, not only for the general thermochemical interest such results would have, but in the belief, also, that the caloric values obtained would throw further light on the chemical relationships of these tissue proteids, and ultimately be of worth in any metabolic study of their syntheses and transformations.

The researches of Stohmann, B. Danilewsky, Rubner, Berthelot and Atwater, and their pupils, have shown that the combustion equivalents of the chemically pure animal proteids thus far studied vary from averages of 5270 calories for gelatin and 5298.8 calories for

pepton, to 5961.3 calories for elastin; with egg albumin, at 5735.2 calories, representing about the mean value.[1] The work of these observers also indicates in a general way that the higher the percentage of carbon in the proteid, the greater its combustion equivalent; the greater the proportion of oxygen, on the other hand, the lower the heat of combustion. Thus elastin, which, we have seen, has the highest equivalent, contains about 55 per cent of carbon and 20 per cent of oxygen; pepton, with a much lower equivalent, contains roughly 50 per cent of carbon and 26 per cent of oxygen; albumin, having an average combustion equivalent, contains approximately 52.5 per cent of carbon and 23 per cent of oxygen.

Considerable variation is to be noted on comparing the figures for calories obtained for the same compound by different observers. This fact may be attributed, however, to different degrees of purity of the products burned, as well as to variations in the accuracy of the methods employed. Thus the caloric value of "ossein" is 5039.9 according to Stohman and Langbein[2] and 5410.4 according to Berthelot and André[3] — a difference of 370.5 calories. But as "ossein" is in strictness a tissue residue, not a pure chemical substance, these variations are not at all surprising.

The only strictly compound proteid investigated by combustion methods thus far is hæmoglobin. Its potential energy appears to be relatively high, varying from 5885.1[2] to 5914[3] calories. The com-

[1] The first of these figures was obtained by Atwater (see foot-note, p. 419). The rest were determined by Stohmann and Langbein, with the improved Berthelot method, and are taken from the table in the Centralblatt für Physiologie for 1892 (vi), p. 157. B. Danilewsky obtained somewhat lower figures for pepton, an average of 4900 calories (Centralblatt für die medicinischen Wissenschaften, 1885, xxiii, p. 678), but as these were derived by the older Thompson-Stohmann process, which was not as accurate as the Berthelot method, the values given by Stohmann and Langbein are probably more trustworthy. Fibroin is the only native proteid thus far studied which has a combustion equivalent lower than that of pepton. According to Stohmann and Langbein it is 4979.6 calories. Berthelot and André found it to be 5095.7 (Centralblatt für Physiologie, 1890, iv, p. 609). An excellent résumé of combustion methods and results is given by Atwater: Methods and results of investigations on the chemistry and economy of food (Bulletin No. 21, Office of Experiment Stations, U. S. Department of Agriculture), 1895, p. 113; also by Bunge: Lehrbuch der physiologischen und pathologischen Chemie, 1894, p. 62, and by Gautier: Leçons de chimie biologique normale et pathologique, 1897, p. 788.

[2] Stohmann und Langbein: Centralblatt für Physiologie, 1892, vi, p. 156.

[3] Berthelot et André: *Ibid.*, 1890, iv, p. 609.

bustion equivalent of milk casein, classified, by some, as pseudonucleoproteid, varies from 5629.2[2] to 5858.3[1] calories.

Of the results thus far obtained in calorimetric experiments the most important for us in this particular connection are those for "chondrin." Stohmann and Langbein have found the combustion equivalent of "chondrin" to be 5130.6 calories;[1] Berthelot and André[2] place it at 5345.8 calories.[3] This difference of 211.8 calories may be attributed to variations in the composition of the product burned, for "chondrin," with approximately 50 per cent of carbon and 28 per cent of oxygen, is a mixture consisting mostly of cartilage gelatin, chondromucoid and chondroitin sulphuric acid. It is almost impossible to make two preparations of the mixture having the same composition and in which the proportions of the components are alike. It is to be observed, however, that, even if the higher figures be accounted more correct, the value expressed by them is still about as low as any thus far determined for animal proteid — even for the *hydrated* forms such as pepton. The lowered potential energy of "chondrin," as well as its lowered percentage of carbon and the raised proportion of oxygen, may be reasonably attributed in great part to the carbohydrate portions of the contained chondroitin sulphuric acid and chondromucoid.[4]

METHOD OF DETERMINATION.

The determinations of heat of combustion in our own experiments were made in a Berthelot bomb calorimeter as modified and improved by Atwater and Blakeslee. Most of the experimental work in this connection was done by Mr. Hawk, in the chemical laboratories of Wesleyan University, the privileges of which were very kindly extended for the purpose by Professor Atwater, to whom we are

[1] STOHMANN und LANGBEIN: *Loc. cit.*

[2] BERTHELOT et ANDRÉ: *Loc. cit.*

[3] B. DANILEWSKI, working with the older and less accurate method, found it to be 4909 calories: Centralblatt für die medicinischen Wissenschaften, 1885. xxiii, p. 678.

[4] The values for heat of combustion of connective tissue collagens have never been determined. For the hydration product of mixed collagens, commercial gelatin, the value is 5,270 calories. ATWATER: Report of the Storrs (Conn.) Agricultural Experiment Station. 1899. p. 92 (Fish gelatin = 5493 calories: B. DANILEWSKY, *loc. cit.*). Cartilage gelatin has not been studied, in this connection. The combustion equivalent of disaccharides averages about 3900 calories; of polysaccharides about 4200 calories.

greatly indebted, also, for many courtesies and much valuable assistance.

Combustions of pure substances of known calorific power were made at intervals to test the apparatus and manipulations. The customary method of ignition, by means of iron wire, was used, and the

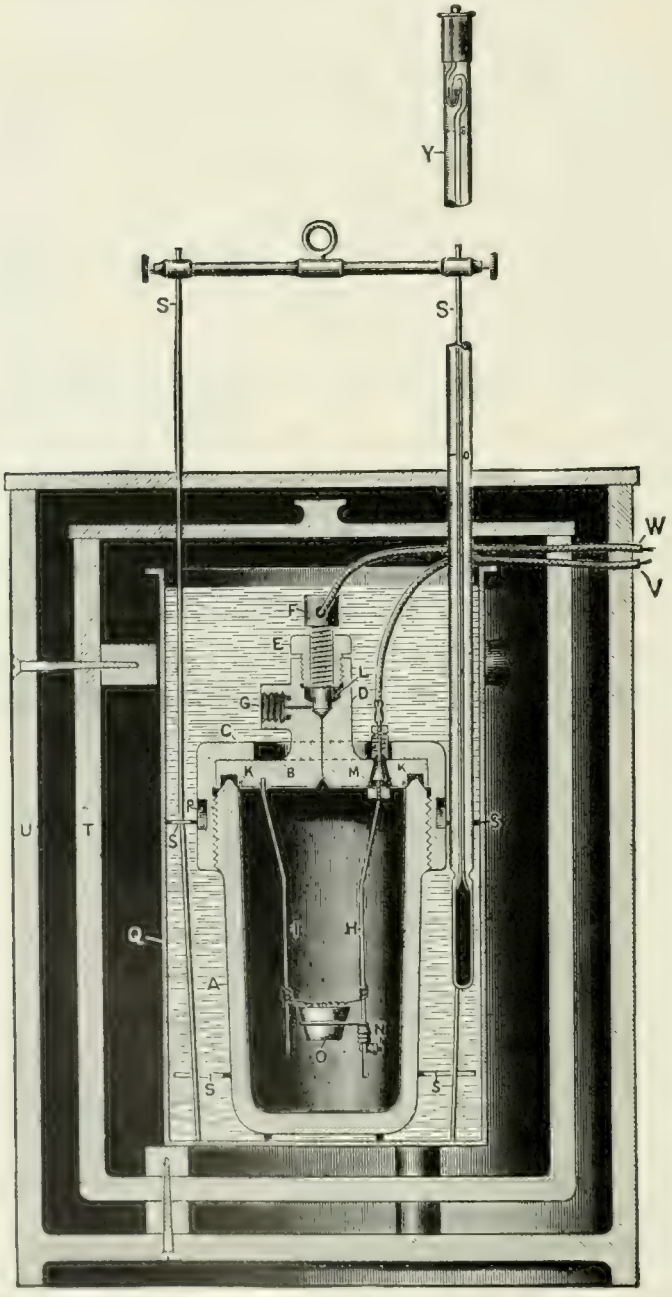


FIGURE 1. — Atwater-Blakeslee bomb calorimeter and accessory apparatus as arranged for combustions. — The platinum lined bomb of steel, holding oxygen and the substance to be burned, is immersed in water contained in a metal cylinder (Q); the latter is surrounded by concentric covered cylinders (T, U) of indurated fibre. Air spaces between the outer cylinders favor retention of heat in the water. The water is kept in motion with the aid of a stirrer (SS) driven by a small electric motor, thus equalizing temperature. Oxygen is forced into the empty bomb through the side passage (G) in the neck (D). Perfect closure of this passage is made by the valve screw (F). The electric current, for fusing the iron wire over the substance to be burned in the capsule (O), is conveyed by the insulated wires (W, V), one of which (W) is connected with the valve screw (F) and thus with one of the platinum wires inside the bomb (I), and the other (V) with the insulated platinum wire (H) which passes through the cover of the bomb. The thermometer is graduated to hundredths of a degree, and is capable of being read to thousandths with a magnifying lens.[1]

[1] For full description see ATWATER and BLAKESLEE: Report of the Storrs (Conn.) Agricultural Experiment Station, 1897, p. 199.

necessary correction made for its heat of combustion. Proper correction was also made for the thermal changes due to oxidation of the nitrogen of the proteid to nitric acid. The quantities of proteid employed in each determination varied from 0.6 to 1.0 gram. Each sample burned completely without special difficulty.

Two of the best of our completely analyzed preparations of osseomucoid were burned in the bomb. Samples of preparations No. 5 and No. 6 (see preceding section) were selected for the purpose. All but one of the tendon mucins employed for the same purpose were prepared and analyzed by Cutter and Gies,[1] and represent the glucoproteids, made by fractional precipitation methods, from both the sheath and the shaft of the tendo Achillis of the ox. The mucin of preparation, "c 8" was made and analyzed several years ago by Chittenden and Gies.[2] The preparations of chondromucoid which we oxidized in the calorimeter were made by Mörner's[3] method, especially for this work. Preparation "a 9" represents the mixed mucoid from three successive extractions of cartilage from the nasal septum of the ox; preparation "b 10" only the glucoproteid in the second extract of a separate portion of cartilage from the same source. Elementary analyses, in duplicate, were made by the methods given on page 403.

EXPERIMENTAL RESULTS.

In the summary on page 422 the figures in duplicate determinations, under "heat of combustion," represent small calories at constant volume per gram of substance dried at 100–110° C. to constant weight; the analytic figures represent elementary composition of perfectly anhydrous substance; complete averages and other data are also included.

DISCUSSION OF DATA.

The striking feature of the results for heat of combustion is the fact that they are uniformly low. The general averages fall far below the figures for potential energy of all the common proteids, including the hydrated forms, and even beneath the smallest equivalent recorded for fibroin (see page 418). This result was naturally to be expected,

[1] CUTTER and GIES: *Loc. cit.* The complete analytic data given here for these preparations anticipate the detailed publication of the results obtained.

[2] CHITTENDEN and GIES: *Loc. cit.*

[3] C. TH. MÖRNER: Skandinavisches Archiv für Physiologie, 1889, i, p. 210.

COMBUSTION EQUIVALENTS AND ELEMENTARY COMPOSITION OF GLUCOPROTEIDS.

Preparation.	Direct determinations. Heat of combustion. Small calories. Per gram.			Average percentage composition of ash-free substance.					Ash.	Averages: Calculated for ash-free substance. Heat of combustion. Small calories.				Carbon.	Oxygen.
				C	H	N	S	O	Per cent.	Per gm.	For subst'nce containing 1 gm. carbon.	Group averages.			
	I	II	Average							X	Y	X	Y	Per cent.	
A. Osseomucoid.															
No. 5.—1	4915	4927	4921	47.78	6.53	11.72	2.50	31.47	0.29	4935	10329				
No. 6.—2	5029	5044	5037	46.53	6.81	11.99	2.55	32.12	0.24	5049	10850	4992	10589	47.16	31.79
B. Tendon mucin.															
a. From shaft 3	4925	4940	4933	47.47	6.68	12.58	2.20	31.07	0.69	4967	10463				
4	4963	4930	4947	47.23	6.56	11.78	1.81	32.61	0.80	4986	10558				
5	4921	4934	4928	47.61	6.60	12.66	1.85	31.18	1.04	4979	10459				
b. From sheath 6	4908	4920	4914	48.92	6.83	12.64	2.80	28.81	0.75	4951	10121				
7	5044	5036	5040	48.25	6.54	12.69	2.34	30.18	1.78	5131	10635				
c. From shaft and sheath 8	5010	5007	5009	48.74	6.46	11.80	2.35	30.65	0.67	5042	10345	5009	10430	48.04	30.75
C. Chondromucoid															
a. Of several extracts 9	4835	4850	4843	46.15	6.51	11.95	2.28	33.11	0.30	4857	10525				
b. Of second extract 10	4895	4888	4892	45.58	6.80	12.38	2.55	32.69	0.34	4909	10769	4883	10647	45.87	32.90
General Averages	4944	4948	4946	47.43	6.63	12.22	2.32	31.40	0.69	4981	10505	4981	10505	47.43	31.40

however, because of the decreased proportion of carbon and nitrogen, and the raised percentage of sulphur and oxygen produced in these compound substances by the union of proteid with carbohydrate and sulphuric acid radicles in their construction. The general average equivalent falls about midway between the figures for calorific value of polysaccharide and albumin.

Very little stress can be laid on the differences shown in the above table for the separate groups, because they are entirely too slight, and quite within the limits of unavoidable experimental error. On the other hand, the group agreement is so decided in the main that further experimental evidence is furnished, we think, of the chemical similarity and close relationship of the three substances, or groups of substances, under examination. It is interesting, also, to find that such differences as are expressed in the group averages run parallel with the fluctuations in amount of carbon and oxygen, the equivalents increasing as the percentage of carbon rises, and falling as the oxygen goes up in proportion.

The above average figures for composition and combustion equivalent are brought into direct comparison below with a similar average given by Stohman and Langbein: — [1]

Investigators.	Substances.	Average percentage composition.					Combustion equivalent. Small calories.
Stohmann and Langbein.	Numerous animal and vegetable proteids; not including mucoids.	C	H	N	S	O	5711
		52.64	7.08	16.00	1.03	23.20	
Hawk and Gies.	Connective tissue glucoproteids only.	C	H	N	S	O	4981
		47.43	6.63	12.22	2.32	31.40	

The general relation of our results to those obtained for other common proteids and albuminous mixtures is so clearly shown in the table [2] of averages on page 424 that further comment is unnecessary.

[1] STOHMANN and LANGBEIN: *Loc. cit.*

[2] Results not our own are selected from those for many substances burned and analyzed by Berthelot and André: *Loc. cit.*

Substance.	Combustion equivalent. Small calories.	Combustion equivalent. Large calories.	Percentage of carbon.	Percentage of oxygen.
	Per gram.	For substance containing 1 gm. of carbon.		
Chondromucoid.	4883	10.65	45.87	32.90
Tendon mucin.	5009	10.43	48.04	30.75
Osseomucoid.	4992	10.59	47.16	31.79
Hæmoglobin.	5914	10.62	55.51	17.62
Egg albumin.	5691	10.99	51 77	24.15
"Ossein."	5414	10.81	50.10	24.60
"Chondrin."	5346	10.54	50.89	23.03
Fish gelatin.	5242	10.80	48.53	25.54
Fibroin.	5097	10.60	48.09	27.41

IV. Summary of Conclusions.

1. A substance, designated as osseomucoid, having the chemical and physical qualities of mucin and chondromucoid, may be extracted from the rib and femur of the ox with lime water. Such extraction may be made most satisfactorily from ossein prepared, in the form of shavings, from bones which have previously been partly decalcified with very dilute acid (0.05–0.5 per cent HCl).

This discovery makes it evident that ordinary compact bone, like the other forms of connective tissue, does contain mucin substance, and further, contrary to Young's deduction, that in the process of ossification the connective tissue matrix is *not* completely removed.

2. The percentage composition of seven preparations of osseomucoid varied between the following extremes, with the subjoined general averages for the seven, also for the four agreeing quite closely and to which particular attention was given in the process of purification: —

	C	H	N	S	O
Extremes:	49.08–46.40	7.42–6.53	14.44–11.72	1.40–2.55	27.92–32.65
Average 1–7:	47.53	6.92	12.85	2.05	30.65
Average 4–7:	47.07	6.69	11.98	2.41	31.85

It is probable that there are two or more glucoproteids in bone, judging from the variations noted in the results for percentage composition.

Osseomucoid does not contain phosphorus. Between 1 and 1.6 per cent of its sulphur may be split off as SO_3 on boiling in dilute hydrochloric acid.

3. The energy liberated on oxidation of the mucin substances, as represented by osseomucoid, tendon mucin, and chondromucoid, is less than that for any other form of proteid except fibroin. The average of twenty duplicate determinations for ash-free substance is 4981 small calories per gram, just midway between the average equivalents for albumin and polysaccharide.

The average potential energy of osseomucoid (4992), tendon mucin (5009), and chondromucoid (4883) is found to be so nearly the same for each substance that additional experimental evidence is furnished of the very close chemical relationship of these connective tissue glucoproteids. Slight and variable differences in the content of carbon and oxygen in these substances appear to account for the minor fluctuations in the figures for combustion equivalent.

The average elementary ash-free percentage composition of the ten samples of typical glucoproteid studied by the combustion method is :

C	H	N	S	O
47.43	6.63	12.22	2.32	31.40

The figures for elementary ash-free composition of the preparations of tendon mucin and chondromucoid studied in this connection agree quite well with those for similar products analyzed several years ago by Mörner and by Chittenden and Gies. The observed analytic variations are comparatively slight, but suggest that tendon and cartilage each contains several closely related mucin substances.

ON THE EXCRETION OF KYNURENIC ACID.
(Second Paper.)

By LAFAYETTE B. MENDEL and EDWARD C. SCHNEIDER.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University.*]

IN an earlier communication[1] the importance and interest attaching to the study of kynurenic acid production in the animal body was pointed out, and the literature on the subject reviewed at some length. The experimental data obtained led the writer to the conclusion that kynurenic acid is formed in connection with proteid katabolism in the dog, and does not owe its immediate origin to putrefactive changes in the intestine. In accord with this view it was observed that a larger excretion of kynurenic acid usually accompanies increased proteid metabolism, whether this condition be brought about by starvation, by ingestion of various proteid foods, or by the action of certain drugs.[2] The significance of various proteids (animal and vegetable) was investigated, and the peculiar influence of gelatin in suppressing the excretion of kynurenic acid emphasized.

A renewed investigation of some of the problems connected with the formation of kynurenic acid has brought further evidence in support of certain conclusions already advanced, and has added new facts regarding the metabolic processes in the dog. The methods employed have been essentially the same as those already described in the first paper. The food materials not specifically discussed below were commercial cracker-dust containing 1.5 per cent of nitrogen and briefly termed *carbohydrate* food; the *fat* used was lard practically free from nitrogen; the *casein* was usually freshly precipitated from skimmed milk and contained from 3.5 to 3.9 per cent of nitrogen; the *gelatin* was a commercial product containing 13.8 per cent of nitrogen; and in addition various mixtures of inorganic salts, including that suggested by I. Munk,[3] were administered when pure foods were fed.

[1] Mendel and Jackson: This journal, 1898, ii, p. 1.

[2] *Cf.* also the recent confirmatory results obtained by Gies after administering tellurium compounds: This journal, 1901, v, p. 191.

[3] I. Munk: Archiv für pathologische Anatomie, 1893, cxxxii, p. 102.

Influence of Fasting.

In the first paper on the excretion of kynurenic acid attention was directed to the occurrence of this substance in the urine of fasting dogs under conditions where body proteid was necessarily being broken down. This observation added support to the view that kynurenic acid originates in the true metabolic processes of the organism, rather than directly in putrefactive changes in the intestine. In the abstract of our paper which appeared in the Centralblatt für Physiologie,[1] the reviewer has taken occasion to question this conclusion. The following experiments are therefore recorded in order to give further evidence of our earlier statements. Dogs of various sizes were confined in cages, and water alone was given to them. The urine was collected after several days' fasting and analyzed. From every dog, except one, kynurenic acid was obtained under these conditions in quantities varying from 12 mgms. to 158 mgms. per day. With reference to the single animal which failed to yield kynurenic acid even after quite prolonged fasting, it ought to be stated that this dog — a somewhat large one — was under observation in the laboratory at intervals for nearly a year, and no kynurenic acid could be isolated from its urine at any time. Neither after excessive meat diet, nor after injections of phlorhizin (which were found to be very effective in other dogs and which produced marked glycosuria in this animal), could even a trace of kynurenic acid be detected. The animal was finally killed; a superficial examination of the internal organs revealed nothing abnormal.

Protocols of the experiments follow :[2]

I. The urine of a medium sized dog was collected on the seventh and eighth days of fasting. The 239 c.c. collected contained **90 mgms.** of kynurenic acid.

II. A dog of 10 kilos fasted for 12 days. The urine (250 c.c.) collected on the last two days contained **338 mgms.** of kynurenic acid.

III. The urine of a medium-sized dog, collected on the sixth day of fasting, contained **21 mgms.** of kynurenic acid.

IV. The urine of a splenectomized dog was collected on the seventh day of fasting. One hundred and eighty-five c.c. contained **267 mgms.** of kynurenic acid. The urine of another fasting splenectomized dog yielded on one day **204 mgms.**

[1] Mendel and Jackson: Centralblatt für Physiologie, 1899, xiii, p. 130.

[2] Almost all of the analytical determinations in this series were made by Dr. Holmes C. Jackson.

V. A splenectomized dog of 11 kilos received several subcutaneous injections of oleum phosphoratum U. S. P. and refused all food during the severe intoxication. He was observed for 17 days. In the urine (250 c.c.) collected during the last four days there were found **187 mgms.** of kynurenic acid.

VI. In a starving dog of 8 kilos the average daily output of nitrogen was found to be 2.6 grams; the excretion of kynurenic acid averaged **56 mgms.** per day. (See Dog T, 16–20).

Influence of Intestinal Putrefaction.

The experimental data upon which the theory of the intestinal origin of kynurenic acid has rested were reviewed in some detail in our first paper. In consideration of the criticism there offered, the

A.—EXPERIMENT WITH CALOMEL.

Day.	Body Weight.	Urine.				Food.
		Vol.	Ethereal sulphate.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.		Grams.		
1	12.6	300	present		0.033	**calomel**, 2.
2	12.2	140	"		trace	" 2.
3	12.0	550	none		0.047	" 2.
4	12.0	200	"		0.053	none.
5	11.8	225	"		0.014	"
6	11.7	410	"		0.012	**calomel**, 1.
7		130	"		0.042	none.
8	11.5	400	"		trace	**gelatin**, 60; carbohydrate, 100.
9	11.4	625			none	" 70; " "
10	11.2	460		11.59	"	" 70; " "
11	11.2	310		8.25	0.045	**casein**, 235; " "
12	11.2	205		5.95	0.052	" 235; " "
13	11.4	325			0.071	" 235; " "
14	11.4	540		11.82	0.132	" 425; " "
15	11.5	660		13.85	0.148	" 410; " "
16	11.8	840		13.54	none	**gelatin**, 58; " "

evidence in favor of this view seemed inadequate. Attention was directed to an experiment of Baumann [1] in which an undiminished excretion of kynurenic acid was observed in a dog in which several days' fasting and repeated doses of calomel had freed the intestine from putrefactive processes, as shown by the absence of ethereal sulphates from the urine. The significance of this single observation seemed of sufficient importance to demand a repetition of the experiment. The plan was essentially the same as in Baumann's experi-

B.—EXPERIMENT WITH CALOMEL.

Day.	Body Weight.	Urine.				Food.
		Vol.	Ethereal sulphate.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.		Grams.		
1	11.8	160	present		0.078	meat, 250.
2	11.7	500	"		trace	calomel, 4.
3	10.9	440	trace	4.02	0.013	" 2.
4	10.6	300	"	3.51	0.110	none.
5	10.3	280	"	3.39	0.095	"
6	10.2	360	"	5.72	0.117	"
7		350	"	3.15	0.106	"
8	10.0	270	"	2.65	0.063	"

ment; the dogs went without food, and calomel was given in gelatin capsules at intervals as indicated in the tables following. Water was freely given. The calomel always produced the typical symptoms, diarrhoea, etc. In two of the animals not even the slightest traces of ethereal sulphates could be detected in the urine by Baumann's method. The Jaffé-Obermayer test for indican also gave negative results. The influence of subsequent feeding of proteid is shown with Dog A, and the typical effects of gelatin in suppressing kynurenic acid excretion are also indicated with this animal.

Morax [2] observed a noticeable diminution in the excretion of

[1] Baumann: Zeitschrift für physiologische Chemie, 1886, x, p. 131.

[2] Morax: Zeitschrift für physiologische Chemie, 1886, x, p. 321.

ethereal sulphates after the administration of iodoform in daily doses of five grams to a dog. Food was not withheld. Haagen [1] repeated this experiment and determined the daily output of kynurenic acid. No decrease was found during a period of four days, contrasted with five preceding days without iodoform. We have likewise repeated this experiment on a dog of eight kilos. (See

C. — EXPERIMENT WITH CALOMEL.

Day.	Body Weight.	Urine.				Food.
		Vol.	Ethereal sulphate.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.		Grams.		
1	16.2				0.072	**meat,** 450.
2	16.2	240	present		0.072	**calomel,** 2.
3	15.6	590	"	8.31	0.166	" 4.
4	14.8	250	none	2.31	0.083	none.
5	14.4	160	"	2.29	0.125	"
6	14.2	150	"	2.11	0.158	"
7	13.9	250		2.02	0.015 [1]	"
8	13.3	220		1.08	0.071	"

[1] A part of the kynurenic acid was lost.

Dog D.) It was necessary to give somewhat smaller doses of iodoform (four grams), and even these proved extremely toxic. There was considerable unabsorbed iodoform found in the fæces, and iodine was found in the urine. The ethereal sulphates in the urine decreased almost one half during the progress of the iodoform administration. Nevertheless the output of kynurenic acid distinctly increased — an effect which we are inclined to attribute to a stimulation of proteid katabolism by the drug, as indicated by the figures for the nitrogen output. It must also be borne in mind that only a part of the ingested food (containing 6.1 grams of nitrogen per day) was retained during the iodoform period.

[1] Haagen: Ueber den Einfluss der Darmfäulniss auf die Entstehung der Kynurensäure beim Hunde; Dissertation, Königsberg, 1887.

D.—EXPERIMENT WITH IODOFORM.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	7.9	125	4.02	0.066	**casein**, 125; carbohyd., 100.
2	7.9	140	5.16	0.125	" 125; " 100.
3	8.0	150	5.59	0.132	" 125; " 100.
4	8.1	200	6.68	0.147	" 125; " 100; **iodoform**, 4.[1]
5	8.0	345	6.84	0.175	" 125; " 100; " 4.[2]
6	7.9	220	5.25	0.192	" 125; " 100; " 4.[3]
7	7.7	450	9.11	0.374	" 35; " 25.

[1] Dog vomited food = 1.5 grams N.
[2] Dog vomited food = 1.3 grams N.
[3] Dog vomited nearly all food.

In contrast with the undiminished kynurenic acid excretion which was obtained after iodoform administration by both Haagen and ourselves are his results with other antiseptics. Haagen determined the daily kynurenic acid output in a dog on an abundant meat diet, during a preliminary period of about a week (in each experiment) and a subsequent period of equal length during which some antiseptic was given. Salol, thymol, and naphthalene were used. The results may be summarized briefly as follows: —

DAILY EXCRETION OF KYNURENIC ACID (HAAGEN).

Intestinal Antiseptic.	Preliminary period.	Antiseptic period.	Decrease.
	Grams.		Per cent.
Thymol . . .	0.603	0.522	13.4
Salol	0.406	0.275	32.0
Naphthalene .	0.432	0.199	54.0
Iodoform . .	0.591	0.624	none

It has already been noted in connection with these experiments that the explanation of the increase in kynurenic acid observed with some of these substances might be sought in a diminished utilization of proteid equally as well as in intestinal factors. The lack of nitrogen determinations in Haagen's experiments made it impossible to give a definite answer in this regard. With this point in mind we have, therefore, repeated the salol and naphthalene experiments. Our salol trial was made on a bitch of 17.7 kilos which received a constant diet of 450 grams of meat (containing 4.07 per cent of nitrogen) and fifty grams of fat daily. The urine was collected in daily periods by the use of a catheter, and the fæces for each period were separated off by means of charcoal and analyzed. A preliminary period of three days was followed by four days in which five grams of salol were given daily. The experiment was concluded with an after-period of three days. The results are summarized below: —

SALOL EXPERIMENT.

(The figures express the daily averages, in grams.)

Analyses.	Fore-period. (3 days.)	Salol-period. (4 days.)	After-period. (3 days.)
Nitrogen in food ingested . . .	18.32	18.32	18.32
Nitrogen eliminated in urine . .	16.13	17.79	15.94
Nitrogen eliminated in fæces .	0.34	0.21	
Kynurenic acid in urine	0.071	trace	0.071

The decrease in kynurenic acid output during the salol period is even more marked than in Haagen's experiment. The nitrogen estimations, furthermore, show that the explanation for this effect is not to be found in any marked change in proteid metabolism, since the relation between nitrogen intake and output is about the same in each period.

Naphthalene produced precisely similar results in another dog. The food was hashed meat; only the urine nitrogen and the kynurenic acid were estimated. Each period consisted of three days.

NAPHTHALENE EXPERIMENT.

(The figures express the daily averages, in grams.)

Diet.	Urine.	
	Nitrogen.	Kynurenic acid.
Meat, 343	11.3	0.134
Meat, 400 Napthalene, 4	14.7	none

Haagen has reported an experiment in which he observed a decrease of 40.9 per cent in the daily output of kynurenic acid when sterilized meat was fed in place of a like quantity of raw meat. Here again there are no data regarding the relative utilization of the diet; the decrease is attributed to diminished activity of putrefactive bacteria during the diet of sterilized food. However, no noticeable difference in the intensity of the indican reaction for the urine of the two periods was observed. We have repeated this experiment also on a bitch of 16.2 kilos. The daily diet consisted of 350 grams of lean meat (13.3 grams N) and 100 grams of carbohydrate food with water. For the second period of three days the fresh meat was thoroughly sterilized by heating repeatedly in an atmosphere of steam. The urine was removed by catheterization.

STERILIZED MEAT EXPERIMENT.

(The figures express the daily averages, in grams.)

Diet.	Urine.	
(3 days.)	Nitrogen.	Kynurenic acid.
Fresh meat, etc. . . .	11.43	0.076
Sterilized meat, etc. .	12.19	0.059

The decrease in the quantity of kynurenic acid excreted during the feeding of the sterilized meat — 22.5 per cent — is somewhat less than that observed by Haagen. The slight difference in the nitrogen output of the two periods points to no explanation of the variations in the production of kynurenic acid.

A review of all the data presented by no means compels to the conclusion that the immediate origin of kynurenic acid is in the putrefactive processes taking place in the intestine. In contrast with the experiments with sterilized meat is the large output of kynurenic acid obtained after feeding sterilized blood fibrin (see Dog T), and the fact that large yields have repeatedly been obtained with pure foods. No differences have been observed between the output after feeding moist casein that has remained about the laboratory for some days, in place of freshly precipitated casein. The constant occurrence of normal quantities of kynurenic acid in the calomel and iodoform experiments, taken in connection with the evidence of diminished intestinal putrefaction as ascertained by the estimation of ethereal sulphates and by the indican reaction in the urine, seems to us more decisive than the results obtained with salol, naphthalene, and thymol. We can offer no definite explanation of the peculiar action of these drugs. In view of their somewhat related composition as organic compounds, it is of course possible that they all combine in some way with an antecedent of kynurenic acid which thus escapes detection. Thus far, however, we have been unable to demonstrate this. Rosenhain[1] obtained marked diminution in kynurenic acid excretion with naphthalene only. Neither he nor Haagen was able to induce kynurenic acid formation from skatol compounds. With indirect methods Capaldi[2] was unable to get evidence of the putrefactive origin of kynurenic acid. We therefore agree, in this connection, with the conclusion expressed by Josephsohn,[3] that the one unquestioned result thus far ascertained is the dependence of the kynurenic acid output on the character of the diet. For our view that the metabolic, rather than putrefactive processes are here involved, further evidence may now be presented.

Influence of Poisons.

Phosphorus. — The experiments with calomel have demonstrated that kynurenic acid may be excreted in noticeable quantity in the absence of putrefactive processes in the intestine. If kynurenic acid arise in the processes incidental to proteid metabolism, we

[1] Rosenhain: Beiträge zur Kenntniss der Kynurensäurebildung im Thierkörper; Dissertation, Königsberg, 1886.

[2] Capaldi: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 87.

[3] Josephsohn: Beiträge zur Kenntniss der Kynurensäure-Ausscheidung beim Hunde; Dissertation, Königsberg, 1898, p. 13.

may reasonably look for an increased production of the acid when these processes become stimulated. Evidence in this direction has already been obtained by previous experiments with phlorhizin.[1] The output of kynurenic acid in these cases was increased simultaneously with the increase in nitrogen and sugar elimination following the administration of the drug. We can now report equally decisive results in phosphorus poisoning. The great rise in proteid metabolism which this condition brings about has been demonstrated by various writers. This increase is, in fact, only paralleled by that in phlorhizin diabetes. "The rise represented by the

E.—EXPERIMENT WITH PHOSPHORUS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.3	125	4.12	0.052	**casein**, 125; carbohyd., 100.
2	8.3	135	4.99	0.068	" 125; " 100; **ol. phosph.**, $\frac{1}{2}$ c.c.
3	8.3	210	5.31	0.074	" 125; " 100.
4	8.2	195	6.62	0.151	" 125; " 100; **ol. phosph.**, $\frac{1}{2}$ c.c.
5	8.0	160	5.29	0.182	none. " "
6	7.8	40	1.14	0.037	"

figures 295, 302, and 287 per cent in phosphorus poisoning in dogs may be compared with 540, 450, 340 and 340 per cent found by Reilly, Nolan, and Lusk,[2] after giving phlorhizin to fasting dogs, although as may be noted the proteid metabolism in diabetes appears somewhat greater."[3]

In our own experiments the dogs were fed (with casein and cracker-dust) as long as possible during the progress of the poisoning. Lo Monaco[4] has found, in contrast with the usual effect of such poisons, that the rise in nitrogen excretion in phosphorus poisoning

[1] Mendel and Jackson: This journal, 1898, ii, p. 24.

[2] Reilly, Nolan, and Lusk: This journal, 1896, i, p. 395.

[3] Ray, McDermott, and Lusk: This journal, 1900, iii, p. 140. The older literature is reviewed in this paper.

[4] Lo Monaco: Archivio di farmacologia e terapeutica, 1896, iv, p. 373.

is proportionately greater in animals receiving food and drink than in fasting ones. According to him, whereas other poisons are more toxic when the organism is weakened by fasting, phosphorus acts more energetically when metabolism is heightened. Our dogs

F.—EXPERIMENT WITH PHOSPHORUS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	20.5	320	5.58	0.138	**casein**, 175; carbohyd., 50.
2	20.2	380	12.42	0.379	" 175; " 100; **ol. phosph.**, 1 c.c.
3	19.9	345	11.80	0.279	" 175; " 100.
4	19.8	255	7.74	0.182	" 175; " 100.
5	19.6	380	14.40	0.439	" 175; " 100; **ol. phosph.**, 1 c.c.
6	19.1	340	12.42	0.325	" 175; " 100; " "
7	18.7	305	9.16	0.257	(Refused food.)
8	17.7	1155	26.05	0.612	" "
9	17.6	900	8.07	0.125	" "
10	16.9	1325	17.62	1.004	" " **ol. phosph.**, $\frac{1}{4}$ c.c.
11	16.8	900	12.87	0.463	" "
12	16.4	495	6.48	0.157	" " **ol. phosph.**, 1 c.c.
13	16.4	550	10.35	0.217	" "
14	15.9	265	4.91	0.096	**casein**, 50; carbohyd., 40.
15	15.6	530	11.37	0.102	" 70; " 70; **ol. phosph.**, 1 c.c.
16	14.9	950	11.50	0.095	" 70; " 70; " "
17	14.6	320	6.49	0.062	(Refused food.)

received subcutaneous injections of the oleum phosphoratum U. S. P., or of strong solutions of phosphorus in olive oil. The results are given in tabular form above. The effect of the phosphorus on Dog E was not very typical; however, bile elements were abundant in the urine until death. With Dog F most characteristic symptoms were observed, as the effects were less acute than in the other

animal. Leucin and tyrosin were found in the urine, and the final autopsy revealed the well-known pathological changes in the liver and alimentary tract. The almost perfect parallelism between nitrogen elimination and kynurenic acid excretion recalls the similar effect noted in phlorhizin poisoning.[1] To the writer such results seem to speak in favor of the metabolic origin of the kynurenic acid.

Sodium oxalate. — Harnack[2] has ascertained that indicanuria may be brought about in man and in the dog by administration of oxalic or sulphuric acid. The most successful effects seem to be produced

G. — EXPERIMENT WITH SODIUM OXALATE.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	12.2	305	5.60	0.005	**casein**, 150; carbohyd., 60.
2	12.2	315	4.23	trace	" 150; " 60; **sod. oxalate**, 0.21.
3	12.4	180	3.80	0.022	" 150; " 60.
4	12.4	130	3.42	0.035	" 150; " 60.
5	12.3	335	9.40	0.102	" 150; " 60.
6	12.2	215	7.12	0.130	" 150; " 60.
7	12.1	250	8.84	0.139	" 150; " 60.
8	12.0	315	6.64	0.077	" 150; " 60.

by the neutral sodium oxalate injected subcutaneously; very small (non-toxic) doses occasionally suffice. The results reported were by no means constant, and occasionally the effects of the oxalate were very tardy in their appearance. Further investigation of this problem is desirable. Harnack has reached the preliminary conclusion that this type of indicanuria is the outcome of tissue or metabolic transformations, rather than intestinal in origin. It seemed of interest, therefore, to study the excretion of kynurenic acid in dogs in which experimental indicanuria of the type indicated had

[1] Mendel and Jackson: This journal, 1898, ii, p. 25. (Dog K.)

[2] Harnack: Zeitschrift für physiologische Chemie, 1900, xxix, p. 205.

been brought about. Harnack has offered no data regarding the extent of proteid metabolism in the course of the oxalate-indicanuria. We have directed attention to this important point by determining the nitrogen content of the urine. Three experiments were made. Comparative tests for indican were made by means of the Jaffé-Obermayer reaction. The doses of sodium oxalate used were comparable with those recommended by Harnack. In each dog the indican reaction became more pronounced after the injection and continued to be increased for from two to five days. In one animal only (Dog G) was an increase in nitrogen and kynurenic acid output observed, and this occurred when the indicanuria had subsided again. This dog received a daily diet of casein and carbohydrate food which ordinarily afforded very little kynurenic acid. In a fasting dog no increase in nitrogen or kynurenic acid output was obtained; and in the third animal, fed on gelatin and carbohydrate, the kynurenic acid excretion was suppressed as it usually is after this diet.

Hydrazine sulphate. — Experiments involving the action of hydrazine sulphate on fasting dogs gave variable results. The effect of the poison was evident in the symptoms produced and in the appearance of allantoïn in the urine, as first described by Borissow.[1] The discrepancies in the analytical data obtained may doubtless be attributed to the experimental difficulties met with. At any rate, they do not yet justify a detailed report.

Influence of Diet.

The intimate relation between the character of the diet and the extent of kynurenic acid excretion was emphasized in the earlier paper by the writer.[2] Schmidt[3] found the quantity to be largest after a meat diet, smaller with milk, and still smaller with bread. It will be observed at once that this order corresponds in a general way with the relative proteid content of the three dietaries. Josephsohn[4] extended this investigation; for every hundred grams of proteid fed he obtained with his animals an excretion of from one hundred and fifty to three hundred and seventy milligrams of kynurenic acid. The relative efficiency in this respect of the diets used

[1] Borissow: Zeitschrift für physiologische Chemie, 1894, xix, p. 499.

[2] Mendel and Jackson: This journal, 1898, ii, p. 2.

[3] Schmidt: Ueber das Verhalten einiger Chinolinderivate im Thierkörper mit Rücksicht auf die Bildung von Kynurensäure; Dissertation, Königsberg, 1884.

[4] Josephsohn: Dissertation, Königsberg, 1898.

is expressed in the following series: egg-albumin with bread, fresh meat, Liebig's extract with bread, desiccated-meat powder, casein, spleen, bread. Thymus was found to give considerably lower results; Josephsohn concludes that the nucleins do not take part in the formation of kynurenic acid and may even check its production from other proteids. It is suggested that the latter action might be attributed to a disinfection of the alimentary tract by the nucleic acid, which is assumed to have marked antiseptic properties. We have already pointed out, however, that the intestinal origin of kynurenic acid is by no means demonstrated; furthermore it may be questioned whether nucleic acid possesses marked action of this character. Josephsohn summarizes his experiments with the statement that the quantity of kynurenic acid excreted is approximately proportional to the extent of proteid decomposition and the quantity of ingested proteid. Independently Mendel and Jackson arrived at a similar conclusion, and they found that kynurenic acid excretion follows the ingestion of both animal and vegetable proteids, as well as proteoses (Witte's peptone). Further investigations are now offered.

Albuminoids. — The absence of kynurenic acid from the urine when gelatin is fed was discussed at length in the earlier paper.[1] A large number of additional experiments on many animals has demonstrated the constancy of this effect. In fact, the influence of gelatin is manifested so quickly that we have repeatedly employed it in marking off successive feeding periods with different diets. A single day's feeding usually suffices to make the urine free from kynurenic acid, so that on the following days the specific action of the product under investigation can manifest itself.

In view of this peculiar deportment of gelatin in metabolism, it became of interest to study the effect of other albuminoids. For this purpose hydrated cartilage (the so-called "chondrin" of older writers) and elastin were fed. The former was prepared by heating the carefully cleaned tracheal cartilages of sheep until solution ensued. The material was eaten in the form of a jelly. The "elastin" consisted of the ligamentum nuchæ of cattle. After removal of connective tissue, the ligaments were cut up finely and heated in water for a few minutes. The material thus prepared contained 7.7 per cent of nitrogen. Mann[2] found in experiments on

[1] Mendel and Jackson: This journal, 1898, ii, p. 21.

[2] Mann: Archiv für Hygiene, 1899, xxxvi, p. 166.

man that elastin is digested in moderate quantities and utilized by the body. Although we fed very large amounts, considerable elastin was utilized by the dogs. Kynurenic acid was not formed either after the elastin feeding or the cartilage diet, with the single exception of one day, as will be seen from the protocols. The results with elastin were also confirmed in a similar experiment on a splenectomized dog. Elastin differs from gelatin in yielding small quantities of tyrosin on decomposition.[1]

H.—EXPERIMENT WITH HYDRATED CARTILAGE.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	13.0	255	5.02	0.019	**casein**, 125; carbohyd., 100.
2	12.9	260	4.13	0.022	" 125; " 100.
3	12.7	220	6.59	0.064	" 125; " 100.
4	12.6	70	..	none	**hydrated cartilage**, 125; carbohyd., 100.
5	12.6	215	5.56	0.030	" " 125; " 100.
6	12.4	120	2.46	none	" " 200; " 100.
7	12.2	355	4.74	"	" " 250; " 100.
8	12.2	185	4.99	0.027	**casein**, 150; carbohyd., 100.

Glycocoll. — Gelatin differs from the ordinary proteids in yielding relatively large quantities (8 per cent) of glycocoll on decomposition; after feeding gelatin to rabbits, Parker and Lusk[2] were unable to demonstrate any unusually large formation of glycocoll in intermediary metabolism. It seemed to us appropriate to investigate the influence of glycocoll on kynurenic acid formation, in view of the peculiar action of gelatin on this function. Thus one possibility which suggested itself was that the glycocoll, formed in excessive amounts, might combine with aromatic radicles of the proteid and prevent their synthesis into kynurenic acid. Two dogs were given

[1] Drechsel: Ladenburg's Handwörterbuch der Chemie, 1885, iii, p. 571; Horbaczewski: Monatshefte für Chemie, 1886, vi, p. 639.

[2] Parker and Lusk: This journal, 1900, iii, p. 472.

I. — EXPERIMENT WITH ELASTIN.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.5	110	2.83	0.029	**casein**, 125; carbohyd., 100; fat, 30.
2	8.6	115	5.24	0.072	" 125; " 100.
3	8.7	135	7.03	none	**elastin**, 200; " 100.
4	8.6	200	12.40	"	" 200; " 100.
5	8.8	245	13.40	"	" 200; " 100.
6	8.9	250	13.40	"	" 200; " 50.
7	8.7	300	18.10	"	" 300.
8	8.7	170		0.043	**casein**, 125; carbohyd., 75.
9	8.7	145		0.085	" 125; " 75.

four and five grams respectively of glycocoll along with a casein diet. No variation in kynurenic acid output was observed. To another dog lithium benzoate was administered along with a gelatin diet. The benzoic acid was excreted as hippuric acid (benzoylglycocoll); as usual no kynurenic acid was obtained.

Ovomucoid. — The somewhat crude product fed was obtained by precipitating with alcohol the concentrated filtrates from large quantities of coagulated diluted egg-white.[1] It contained 10.6 per cent of nitrogen. In the absence of references to its decomposition products, 15 grams were decomposed by boiling for several days with three per cent HCl; small quantities of tyrosin were isolated. The effects of the feeding experiment were pronounced.

Thymus. — Josephsohn's thymus feeding experiments have already been referred to. Similar experiments which we have made confirm his results. Desiccated thymus powder prepared by Armour and Co. was fed. It contained 13.8 per cent of nitrogen. In some of the experiments a gelatin diet was made to precede the thymus

[1] Cf. C. Th. Mörner: Zeitschrift für physiologische Chemie, 1893, xviii, p. 525.

J.—EXPERIMENT WITH OVOMUCOID.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	12.3	300	3.86	0.059	**casein**, 125; carbohydrate, 100.
2	12.4	265	4.56	none	**ovomucoid**, 47; " 100.
3	12.4	300	4.82	"	" 47; " 100.
4	12.2	225	2.80	"	" 47; " 100.
5	12.2	240	3.39	0.007	**casein**, 125; " 100.
6	12.1	215	3.44	0.012	" 125; " 100.
7	12.2	165	2.04	0.007	" 125; " 100.

K.—EXPERIMENTS WITH THYMUS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	11.4	290	6.75	0.067	**casein**, 175; carbohyd., 100.
2	11.8	260	6.66	0.104	" 175; " 100.
3	11.8	295	5.97	0.111	" 175; " 100.
4	11.8	150		0.020	" 75; " 100; **thymus**, 29.
5	12.1	215	5.42	0.043	" 75; " 100; " 29.
6	12.0	200	4.74	0.032	" 75; " 100; " 29.
7	12.3	250		0.113	" 175; " 100.
8	12.2	..	7.70	0.060	" 125; " 100.
9	12.3	300	7.70	0.060	" 125; " 100.
1	12.3	275	4.33	none	**gelatin**, 36; carbohyd., 100.
2	12.4	210	5.00	"	**thymus**, 39; " 100.
3	12.4	305	5.59	"	**gelatin**, 36; " 100.

feeding, in order to exclude the effect of the previous proteid diet. The kynurenic acid excretion was either suppressed or greatly diminished in every instance. This was not due to any deficient absorption of the nitrogenous food, as the figures demonstrate. (See Dogs K, L.) In man we have observed no diminution of intestinal putrefaction during thymus feeding and find no occasion for accepting Josephsohn's theory of a possible antiseptic action of the nuclein food.[1]

L.—EXPERIMENTS WITH THYMUS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.7	225	7.45	0.212	**casein**, 175; carbohydrate, 70.
2	8.7	...	6.27	0.184	" 175; " 70.
3	8.7	375	6.27	0.184	" 175; " 70.
4	8.6	225	3.39	0.041	**thymus**, 35; " 70.
5	8.7	465	5.24	0.063	" 30; " 70.
6	8.7	390	4.65	0.047	" 35; " 70.
7	8.7	160	4.60	0.121	**casein**, 100; " 70.
8	8.6	180		0.024	**gelatin**, 40; " 70.
9	8.3	215	7.45	trace	" 35; " 70.
10	8.0	175	5.83	none	" 35; " 70.
1	9.0	185	7.70	0.077	**meat**, 250; carbohydrate, 100.
2	9.1	295	8.22	none	**gelatin**, 40; " 100.
3	9.0	600	5.64	trace	**thymus**, 50; " 100.
4	8.8	510	4.41	none	" 50; " 100.
5	8.9	510	5.63	trace	" 40; " 100.
6	8.9	180	6.15	0.321	**fibrin**, 120; " 100.

[1] That thymus feeding does not diminish intestinal putrefaction, has been shown by Weintraud and by Lewin. See Zeitschrift für klinische Medicin, 1901, xlii, p. 384.

Pancreas and lymphatic glands. — The diminution of kynurenic acid excretion observed during thymus feeding led us to try pancreas and lymphatic glands. These resemble the thymus in yielding allantoïn and an increased uric acid output in both the dog and the cat.[1] Undoubtedly, however, both glands contain more of the simple proteids than the thymus. Good yields of kynurenic acid were obtained with each, as the protocols show. (See Experiments M.)

M.—EXPERIMENTS WITH PANCREAS AND LYMPHATIC GLANDS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	12.4	305	5.59	none	**gelatin**, 36; carbohyd., 100.
2	12.4	245	5.89	0.190	**pancreas**, 290; " 100.
3	12.6	240	5.74	0.123	" 290; " 100.
4	12.8	195		0.092	" 290; " 100.
5	12.8	205		0.069	" 290; " 100.
6	12.8	175		0.010	**casein**, 125; " 100.
7	12.7	185	4.09	0.022	" 125; " 100.
8	12.7	155	4.32	0.029	" 125; " 100.
1	7.9	175	5.83	none	**gelatin**, 35; carbohyd., 70.
2	7.9	165	4.54	0.150	**lymphatic glands**, 200; carbohyd., 70.
3	8.1	180	6.69	0.181	" " 200; " 70.
4	8.2	150	5.81	trace	**gelatin**, 40; carbohyd., 100.

Vegetable proteid. — Kynurenic acid has frequently been found in the urine after a diet of bread. Mendel and Jackson first showed that the crystallized vegetable proteid edestin, prepared from hempseed, calls forth an excretion of kynurenic acid equally as large as that following ingestion of blood-serum proteids. We have since fed the vegetable proteid amandin, obtained from the sweet almond.

[1] Mendel and Brown: This journal, 1900, iii, p. 261; Mendel and Jackson: This journal, 1900, iv, p. 166.

This substance, which was isolated and described by T. B. Osborne,[1] is particularly of interest because of its very high nitrogen content. Our preparation, which we owe to the kindness of Dr. Osborne, contained 18.9 per cent. It will be observed in the tables that this vegetable proteid also readily yields kynurenic acid in metabolism; the value of the pure vegetable proteids in nutrition has lately been emphasized from other standpoints.[2]

N.—EXPERIMENTS WITH VEGETABLE PROTEID.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	cc.	Grams.		
1	8.0	335	7.33	0.006	**gelatin**, 40; carbohydrate, 100.
2	7.9	125	5.66	0.097	**amandin**, 30; " 100.
3	8.0	115	4.83	0.014	**gelatin**, 35; " 100.
1	8.3	125	5.93	none	**gelatin**, 35; carbohydrate, 100.
2	8.3	125	6.18	0.081	**amandin**, 30; " 100.
3	8.3	150	6.02	none	**gelatin**, 35; " 100.

Cleavage products of the proteids. — After investigation of the rôle of various foodstuffs in the production of kynurenic acid, attention was directed to the cleavage products of the proteids. That the ingestion of proteoses may be followed by kynurenic acid excretion has already been demonstrated with Witte's peptone. We have conducted a more systematic series of experiments with the cleavage products of casein which were obtained by (*a*) gastric digestion, (*b*) tryptic digestion, (*c*) decomposition with acid. The gastric products were prepared by treating casein for two days at 40° C. with a very active pepsin-hydrochloric acid solution containing 0.4 per cent HCl. The fluids were neutralized with sodium hydroxide, and after removal of the neutralization precipitate and paranuclein

[1] Osborne and Campbell: Connecticut Agricultural Experiment Station Report, 1895, p. 289.

[2] Cf. Loewy and Pickardt: Deutsche medicinishe Wochenschrift, 1900, No. 51.

formed, were concentrated to a thick syrup. Mixed with cracker-dust this formed a viscid mass which was readily eaten by the dogs. The tryptic products were similarly prepared after digestion with chloroform-water extracts of fresh pancreas in a dilute sodium carbonate solution. Hydrochloric acid was used to neutralize the digestive mixture. In the hydrolysis with acid the casein was boiled with four per cent sulphuric acid until the mixture no longer gave any biuret reaction. The acid was then removed with barium carbonate and the dissolved barium carefully precipitated with dilute sulphuric acid. The solution was then concentrated and fed as in the other cases.

The products prepared in the manner just outlined represent various stages in the proteolysis of the casein. The decomposition with acid was carried to a point where true proteids no longer were present; in conformity with present views the gastric products may be looked upon as standing nearest to the original proteid. The experimental results obtained with these different materials are given in the appended tables.

A survey of the results presented indicates (as the earlier experiments with Witte's peptone led us to expect) that the ingestion of the products of the gastric digestion of casein is followed by an excretion of kynurenic acid quite as marked as is obtained with the original proteid. With tryptic products similar effects were observed, although in two cases there was a tendency towards a diminished output. While it may be a coincidence merely, it is worthy of note that the tryptic digestions in preparation for these two experiments were continued longer than in the other cases — three and four days, as contrasted with almost two days. From the products of the tryptic digestion of egg-albumin fed to Dog O, considerable of the leucin and tyrosin had been removed; a relatively higher content of soluble proteid in the mixture may perhaps account for the favorable results obtained with this material. In two instances the insoluble material which usually separates out from digestive mixtures was fed with negative results. With the non-proteid cleavage products obtained by hydrolysis with acid the results were decisive. No kynurenic acid was excreted. It would be interesting to study the effect of the products of a tryptic digestion prolonged until all substances giving the biuret reaction had been broken down.

It remains to consider the rôle of some of the individual products of proteolysis. *Heteroalbumose* was at once selected as particularly

O.—EXPERIMENT WITH CLEAVAGE PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	9.0	160	6.99	0.072	**casein**, 125; carbohyd., 100.
2	8.9	150	7.74	0.062	" 125; " 100.
3	8.8	175	5.76	0.076	" 125; " 100.
4	8.7	140	5.92	0.079	" 125; " 100.
5	8.7	210	6.82	0.063	**trypsin products**; carbohyd., 100.
6	8.7	300	6.53	0.053	" " " 100.
7	8.6	125	4.83	0.040	" " " 100.
8	8.6	125	3.39	0.035	**casein**, 125; " 100.
9	8.6	135	6.53	0.051	" 125; " 100; fat, 20.
10	8.7	125	5.38	0.062	" 125; " 100; " 30.
11	8.6	235	6.16	0.057	**pepsin products**; " 100; " 30.
12	8.6	300	7.43	0.091	" " " 100; " 30.
13	8.5	250	5.50	0.050	" " " 100; " 30.
14	8.5	75		none	**undigested residue**; " 100; " 30.
15	8.5	120	4.77	0.012	**casein**, 125; " 100; " 30.
16	8.5	110	2.83	0.029	" 100; " 100; " 30.
17	8.6	115	5.24	0.072	" 100; " 100; " 10.
18	8.6	110	5.42	0.055	" 100; " 100; " 10.
19	8.5	110	3.19	none	**H_2SO_4 products**; " 100; " 10.
20	8.5	230	3.49	"	" " " 100; " 10.
21	8.5	110	5.24	"	" " " 100; " 10.
22	8.3	110	4.69	0.075	**casein**, 125; " 100.
23	8.3	140	7.90	0.122	" 160; " 100.
24	8.4	135	6.05	0.121	**trypsin products** (from albumin); " 100.
25		190	8.32	0.142	**trypsin products** (from albumin); " 100.
26	8.3	265	7.06	0.029	**casein**, 125; " 100.
27	8.3	185	4.30	0.066	" " " 100.
28		200	6.38	0.066	" " " 100.

P.—EXPERIMENTS WITH CLEAVAGE PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.6	170	5.96	0.066	**casein**, 170; carbohydrate, 100.
2	8.7	150	5.47	0.016	" 170; " 100.
3	8.7	145	4.63	0.011	" 170; " 100.
4	8.8	360	5.52	trace	" 170; " 100.
5	8.8	380	4.95	0.014	**pepsin products**; " 100.
6	8.6	300	4.99	0.013	" " " 100.
7	8.6	300	4.52	0.015	" " " 100.
8	8.6	100		0.013	**casein**, 125; " 100.
1	10	170	8.84	0.058	**casein**, 125; carbohydrate, 75.
2	10	125	4.21	trace	" 125; " 75.
3	10	110	6.48	0.040	" 125; " 75.
4	10	135	7.01	0.019	" 150; " 75.
5	10	350	9.61	0.006	**trypsin products**; " 100.
6	10	370	[1]	0.009	" " " 100.
7	10	180	[1]	0.005	**casein**, 150; " 100.

[1] Fæces in urine.

deserving of study. It is in many respects the best defined of all the proteoses. Its distinctive place in nutrition has only recently been pointed out by Blum,[1] who was unable to maintain nitrogen equilibrium in a dog fed on heteroproteose, whereas the caseoses yielded a perfect nitrogen balance. Our heteroalbumose was in part prepared from Witte's peptone by Pick's method as described by Blum; part of it was prepared by us from fibrin digestions. It contained 14.9 per cent of nitrogen. The remainder of the albumoses

[1] Blum: Zeitschrift für physiologische Chemie, 1900, xxx, p. 15; Cf. also Chittenden, Mendel, and Henderson: This journal, 1899, ii, p. 142.

Q.—EXPERIMENTS WITH CLEAVAGE PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.8	290	8.55	0.058	**meat**, 250; carbohyd., 100.
2	9.1	210	7.28	0.073	" 250; " 100.
3	9.1	260	9.72	0.075	" 250; " 100.
4	9.0	190	7.74	0.091	" 250; " 100.
5	9.0	235	7.74	0.005	**trypsin products** (of meat); carbohyd., 100.
6	9.0	345	6.72	none	**trypsin products** (of meat); " 100.
7	9.0	185	7.70	0.077	**meat**, 250; " 100.
1	9.1	200	5.28	0.053	**casein**, 175; carbohyd., 100.
2	8.9	130	5.18	0.059	" 150; " 100.
3	8.9	160	5.89	0.048	" 150; **glycocoll**, 4; carbohyd., 100.
4	8.9	215	6.82	0.059	" 150; carbohyd., 100.
5	8.8	135	4.98	none	**H_2SO_4** products; " 100.
6	8.7	130	4.18	"	" " " 100.
7	8.7	210	7.23	0.083	**casein**, 175; " 100.

composing the Witte's peptone after removal of the heteroalbumose with alcohol, was also prepared for feeding. It contained considerable protoalbumose. As hemialbumose we have designated mixed proteoses obtained by treating egg-albumin with dilute sulphuric acid for a short time.

The general plan of the following experiments was to feed gelatin to the dogs until the excretion of kynurenic acid practically ceased. The cleavage product was then given between two gelatin days.

In every instance the heteroalbumose occasioned a very marked output of kynurenic acid, no less than half a gram being excreted by a small dog (S) after administration of 35 grams of the proteose; in the same animal 83 mgms. were excreted after ingestion of only nine grams of heteroproteose, despite simultaneous feeding of twenty

R.—EXPERIMENT WITH CLEAVAGE PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	18.8	340		0.046	**casein**, 125; carbohyd., 100.
2	18.7	415	9.35	0.115	" 150; " 100.
3	18.3	155	6.04	0.061	" 150; " 100.
4	18.1	250	8.54	0.043	**trypsin products**; carbohyd., 100; fat, 40.
5	18.2	255	5.57	0.016	" " " 100; " 40.
6	18.2	300	6.67	0.010	" " " 100; " 40.
7	18.2	345	7.02	0.019	**casein**, 175; " 100; " 40.
8	18.2	300	7.49	0.008	" 175; " 100; " 40.
9	18.1	310	6.92	0.029	" 175; " 100; " 40.
10	18.0	250	6.08	0.012	" 175; " 100; " 40.
11	17.9	330	7.85	0.032	**pepsin products**; " 100; " 40.
12	17.9	350	7.58	0.020	" " " 100; " 40.
13	18.0	270	7.94	none	" " " 100; " 40.

grams of gelatin and one hundred grams of cracker-dust. The *residue* of the Witte's peptone after removal of heteroproteose was also somewhat effective in calling forth an excretion of kynurenic acid (See Dog T.) We therefore turned to the mother proteid from which this commercial product is reported to be prepared. Washed *fibrin* was fed (after sterilization) in moist form. An abundance of kynurenic acid was excreted during this diet, and far exceeded the yield after ingestion of casein.

The excretion of small quantities of kynurenic acid after *leucin* feeding is noteworthy. Whether it is to be interpreted as a starvation effect comparable with those described earlier in the paper, or whether it is perhaps a specific action, we are at present unable to determine with certainty. The leucin was separated from pancreatic digestions and repeatedly purified by recrystallization. It contained 10.6 to 10.8 per cent of nitrogen (theory 10.6 per cent). The quan-

S.—EXPERIMENT WITH DIGESTION PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.1	170	7.82	none	**gelatin**, 40; carbohyd., 100.
2	8.1	175	7.72	"	" 35; " 100.
3	8.1	160	6.20	0.446	**heteroalbumose**, 33; " 100.
4	8.1	70		0.077	**gelatin**, 35; " 100.
5	8.1	180	7.99	none	" 35; " 100.
6	8.1	135	5.31	0.079	**hemialbumose**, 35; " 100.
7	8.1	120	5.00	0.031	**gelatin**, 35; " 100.
8	8.1	195	9.78	none	" 35; " 100.
9	8.1	110	4.67	"	" 27; " 100; **tyrosin**, 1.34.
10	8.1	95	4.31	"	" 35; " 100.
11	8.2	195	9.16	"	" 32; " 100.
12	8.1	170	5.13	0.043	**leucin**, 27; " 100.
13	7.9	115	5.37	none	**gelatin**, 35; " 100.
14	8.0	95	3.79	0.049	**casein**, 125; " 100.
15	8.1	130	4.84	0.106	" 125; " 100.
16	8.1	125		0.093	" 125; " 100; **tyrosin**, 2.2.
17	8.1	275	7.76	0.277	" 125; " 100; " 2.
18	8.1	150	5.41	0.120	" 125; " 100.
19	8.1	245	6.30	0.230	" 125; " 100.
20	8.0	400	10.38	0.342	" 125; " 100.
21	8.0	90	3.90	0.035	**gelatin**, 40; " 100.
22	7.9	355	7.33	none	" 40; " 100.
23	7.9	175	6.54	0.083	**heteroalbumose**, 9; " 100; **gelatin**, 20.
24	8.0	335	7.33	0.007	**gelatin**, 40; " 100.

T.—EXPERIMENT WITH DIGESTION PRODUCTS.

Day.	Body Weight.	Urine.			Food.
		Vol.	Nitrogen.	Kynurenic acid.	Grams.
	Kilos.	c.c.	Grams.		
1	8.9	180	6.15	0.321	**fibrin**, 120; carbohyd., 100.
2	8.9	90	4.21	0.226	" 100; " 100.
3	8.6	180	4.70	0.223	" 97; " 100.
4	9.1	200	5.28	0.053	**casein**, 175; " 100.
5	8.9	130	5.17	0.060	" 150; " 100.
6	8.7	210	7.23	0.083	" 175; " 100.
7	8.7	255	7.04	none	**gelatin**, 40; " 100.
8	8.7	145	5.59	0.321	**heteroalbumose**, 37; carbohyd., 100.
9	8.5	150	6.55	0.009	**gelatin**, 40; carbohyd., 100.
10	8.5	105	4.69	0.222	**residue from Witte**, 37; carbohyd., 100.
11	8.5	210	6.33	none	**gelatin**, 35; carbohyd., 100.
12	8.5	140	4.04	0.023	" 5; " 100; **leucin**, 23.
13	8.4	215	6.30	none	" 35; " 100.
14	8.4	160	5.69	"	" 25; " 100; **tyrosin**, 3.
15	8.3	125	5.93	"	" 35.
16	8.3	130	2.38	0.022	none.
17	7.9	100	2.54	0.051	"
18	7.7	80	2.77	0.056	"
19	7.5	70	2.91	0.048	**tyrosin**, 2.
20	7.4	85	2.47	0.052	none.

tities of both leucin and tyrosin fed were made comparable with the amount yielded by sufficient casein to maintain nitrogen equilibrium in dogs of the size used.[1]

Our experiments with *tyrosin* have only served to confirm the

[1] Cf. R. Cohn: Zeitschrift für physiologische Chemie, 1896, xxii, p. 153.

previous observation of Hauser[1] and Solomin.[2] Hauser introduced two grams of tyrosin into the stomach of a dog which excreted no kynurenic acid, without producing any effects; the same outcome followed intravenous injection of one gram in the same animal. Solomin reports a similar negative result. The importance of the problem led us to repeat the trials under varied conditions. Tyrosin was given during (1) starvation, (2) casein diet, (3) gelatin diet. As will be seen in the protocols, the excretion of kynurenic acid peculiar to the conditions referred to was in no way altered. No synthesis could be demonstrated. We can no longer point to the absence of tyrosin-forming aromatic groups in the proteid molecule as a satisfactory explanation of the disappearance of kynurenic acid during gelatin feeding; for on the one hand tyrosin and gelatin together fail to yield kynurenic acid, and on the other hand according to Pick,[3] heteroalbumose, which readily leads to an abundant output of the acid, is extremely deficient in tyrosin- and indol-yielding groups. Furthermore we have shown that the combined cleavage products of casein fail to yield kynurenic acid in metabolism despite the presence of tyrosin among these products.

Influence of Various Organs.

The liver. — In an interesting research Jacoby[4] has pointed out how the study of the autodigestion of the liver promises to throw light upon the intermediate processes of nutrition. Since our studies lead us to look upon kynurenic acid as a product of proteid metabolism, we have tried first of all to find traces of this compound in the liver of the dog. Two methods were employed. (1) A liver was finely comminuted and extracted with dilute ammonia. The solution was then heated to boiling and filtered. This process was repeated several times with the residue, and the filtrates were all united, concentrated to a small volume and examined by Capaldi's method for kynurenic acid. (2) Two fresh, comminuted dog livers were allowed to digest separately for a day with chloroform-water. Considerable material passed into solution, as autolysis ensued. A

[1] Hauser: Archiv für experimentelle Pathologie und Pharmakologie, 1895, xxxvi, p. 3.

[2] Solomin: Zeitschrift für physiologische Chemie, 1897, xxiii, p. 501.

[3] Pick: Zeitschrift für physiologische Chemie, 1899, xxviii, p. 261.

[4] Jacoby: Zeitschrift für physiologische Chemie, 1900, xxx, p. 149.

little ammonia was added; the mixture was filtered and further treated as in (1). One of the livers came from Dog F, which was treated with phosphorus oil and had excreted kynurenic acid in considerable quantities. The results were all negative.

The spleen. — This organ apparently takes no important part in the production of kynurenic acid. Determinations of the output have repeatedly been made in the urine of splenectomized animals under various conditions of nutrition, and no diminution in the excretion of kynurenic acid or uric acid has ever been observed.[1]

The pancreas. — The urine of a dog from which the pancreas had been completely extirpated by Sandmeyer's method[2] was removed from the bladder about thirty-six hours after the operation. The secretion, 100 c.c., contained 62 mgms. of kynurenic acid.

Summary.

Kynurenic acid is regularly found in the urine of fasting dogs. It is always present when the putrefactive processes in the intestine have been completely checked by administration of calomel. After administration of iodoform, intestinal putrefaction is diminished, proteid metabolism is stimulated and the excretion of kynurenic acid also augmented. With salol and naphthalene the output is checked, without any marked decrease in the quantity of nitrogen excreted. Similar results were obtained with sterilized meat in one case.

Poisons like phosphorus and phlorhizin, which greatly stimulate proteid katabolism, occasion a pronounced increase in the output of kynurenic acid. Less constant results were obtained with sodium oxalate and hydrazine sulphate.

The influence of the diet on kynurenic acid production has been investigated further. Kynurenic acid is not obtained after feeding elastin, hydrated cartilage, ovomucoid, or thymus alone. These substances produce effects comparable to that of gelatin. The ingestion of pancreatic and lymphatic glands, blood-fibrin and amandin — a pure vegetable proteid — leads to kynurenic acid excretion.

The rôle of proteid cleavage products obtained by hydrolysis with pepsin, trypsin, and sulphuric acid has been studied. When proteids

[1] Cf. Mendel and Jackson: This journal, 1900, iv, p. 163; also Experiments IV and V, pp. 428–429.

[2] Sandmeyer: Zeitschrift für Biologie, 1892, xxix, p. 86.

are broken down beyond the stage where products giving the biuret reaction are present, the organism no longer responds in the usual way. Kynurenic acid is not formed. Proteose feeding is accompanied by a large excretion of kynurenic acid. Glycocoll apparently does not interfere with its elaboration. Attempts to obtain evidence of the synthesis of kynurenic acid from tyrosin under various conditions failed.

The influence of various organs in kynurenic acid production has been considered.

A PRELIMINARY REPORT ON THE ACTIVE PRINCIPLE OF THE SUPRARENAL GLAND.

By T. B. ALDRICH, Ph.D.

[*From the Biological Department of the Scientific Laboratory of Parke, Davis, & Co., Detroit, Mich.*]

THE most recent and in many respects the most important contribution to our knowledge of the active principle of the suprarenal gland, although not exhaustive, is from Dr. Jokichi Takamine[1] who has isolated the blood-pressure-raising principle of the gland in a stable and pure crystalline form, by a method which he claims to be entirely different from any yet employed. To this body which is very active in raising the blood pressure, he has given the name "Adrenalin."

Adrenalin he describes as a micro-crystalline substance occurring in at least five crystalline forms according to the kind and condition of the solvent used. It is perfectly stable when dry, and melts under decomposition at 207° C. It has a slightly bitter taste, and leaves a numbed feeling on the tongue. The hot saturated aqueous solution deposits crystals on cooling. All aqueous solutions on standing turn from a rose color to red and eventually brown. Acids dissolve it readily. The color reactions, as observed by the various workers in this field, are given for the most part in an intensified manner. None of the following alkaloidal reagents gives a precipitate with adrenalin (presumably using the acid solution); mercuric potassium iodide; picric acid; tannic acid; phosphomolybdic acid; phosphotungstic acid; mercuric chloride: potassium bichromate and potassium chloride. The salts of hydrochloric and sulphuric acids are very soluble in water, and hence difficult to obtain in crystalline form. The substance is very sensitive to oxidizing agents, especially in the presence of alkalies.

The physiological activity of the pure product is astounding; one drop of an aqueous solution of the chloride having the strength of 1 : 10,000 is capable of blanching the normal conjunctiva within thirty to sixty seconds; while 0.000001 gram per kilo of body

[1] Takamine: Therapeutic Gazette, 1901, p. 221.

weight, injected intravenously, raises the blood pressure 14 mm. of mercury. From this it can readily be calculated that as little as 0.000002 gram or less used in a similar manner is sufficient to produce distinct physiological effects in an adult.

In the summer of 1900 the author succeeded in obtaining from the suprarenal glands a very small quantity of a semi-crystalline substance which possessed marked physiological activity when injected intravenously into animals, and which gave the characteristic color reactions with ferric chloride, etc., described by the various workers in this field. A few months later a larger quantity of this same body was obtained in a distinctly crystalline and pure condition. It was now possible to investigate the substance in a preliminary way, and to make a combustion analysis. Before this work could be completed, however, Takamine presented a preliminary paper before the Society of Chemical Industry at its meeting in New York City the latter part of January. From this communication it was learned, by a comparison of the physical and chemical properties of the two bodies, obtained independently of each other, that they were practically the same. Further study confirms their identity — they correspond in practically every respect.

The method used by the author is briefly the following. The finely divided glands are repeatedly extracted with water acidulated with acetic acid at a temperature sufficiently low to prevent any appreciable coagulation of proteid. The united extracts are then heated to boiling and, after cooling, the coagulated proteids removed by filtration. The filtrate is then evaporated *in vacuo* at a temperature of 45° C. to a small volume and after cooling neutral lead acetate [1] is added to complete precipitation. The lead precipitate is removed by filtration and the excess of lead removed from the filtrate with sulphuretted hydrogen. This filtrate is then evaporated *in vacuo* to a small volume and precipitated with from four to five times its volume of 94 per cent alcohol, filtered, and the alcoholic filtrate evaporated to a very small bulk or even to dryness, taken up with water and filtered. To this filtrate an alkali, preferably ammonia, is added, whereby in a short time the active principle is precipitated, usually in the form of

[1] Although neutral lead acetate was used in my first process, I found later that this could be dispensed with, and alcohol (94 per cent) used to remove the greater part of the inert substances. However, it was found several times that the most active and purest specimens were obtained when lead acetate was used, the lead no doubt removing some other principles as well as inorganic substances.

small lumps with radial markings, often however in the form of individual crystals, the form depending on various factors. The active principle is washed by decantation with very dilute ammonia water, placed upon the filter, washed with cold water until the alkali is removed and dried in the air or *in vacuo*. In all the steps it is desirable to work as far as possible in an atmosphere free from oxygen, as the active principle is very sensitive towards oxidizing agents, especially in a neutral or alkaline solution.

The product obtained from the first experiments contained a trace of ash, but later specimens were obtained which were practically free from ash, and finally a specimen which contained no ash [1] was prepared from Takamine's crude product. A portion of a specimen which was obtained in a very pure form by the author and which was used in part for the combustion analyses given below, has retained its original yellow or almost white color for almost six months, while losing none of its physiological activity. This specimen, which consisted of individual crystals, was shown by the blood-pressure experiments to be the purest specimen, so far as physiological activity is a measure of purity, that has come under our observation.

Below are given some combustion analyses made by the author with Takamine's purified product and with that prepared by the author's process. Although not final they may throw some light on this important subject. Both specimens were dried for some time *in vacuo* over sulphuric acid and contained no weighable amount of ash.

TAKAMINE.

I. 0.2972 gm. of the substance gave 0.6332 gm. CO_2 and 0.1913 gm. H_2O, or 58.11 per cent C and 7.17 per cent H.

II. 0.2696 gm. of the substance gave 0.5728 gm. CO_2 and 0.1755 gm. H_2O, or 57.95 per cent C and 7.23 per cent H.

A nitrogen determination from the same substance gave the following results: —

I. 0.2438 gm. of substance gave 17.2 c.c. of N at 22° C. and under a barometric pressure of 742 mm. = 7.79 per cent.

II. 0.2435 gm. of substance gave 16.2 c.c. of N at 17.5° C. and under a barometric pressure 744 mm. = 7.53 per cent N.

[1] This ash-free specimen was prepared for me by Mr. Beckwith of this laboratory.

From these figures we have the following results : —

	I.	II.	Average.
C	58.11	57.95	58.03
H	7.17	7.23	7.20
N	7.79	7.53	7.66
O	26.93	27.29	27.11
	100.00	100.00	100.00

ALDRICH.

I. 0.1667 gm. of the substance gave 0.3554 gm. CO_2 and 0.1102 gm. H_2O or 58.15 per cent C and 7.32 per cent H.

II. 0.2056 gm. of the substance gave 0.4344 gm. CO_2 and 0.1347 gm. H_2O or 57.64 per cent C and 7.34 per cent H.

A nitrogen determination from the same substance gave the following results : —

I. 0.1852 gm. of the substance gave 12.5 c.c. N at 21° C. and under a barometric pressure of 744 mm. = 7.49 per cent N.

From these figures we have the following data : —

	I.	II.	Average.
C	58.15	57.64	57.89
H	7.32	7.34	7.23
N	7.49	7.52	7.50
O	27.04	27.50	27.27
	100.00	100.00	99.99

A comparison of these analytical data shows that the two substances obtained are identical, and using them as a basis for calculating an empirical formula the simplest body obtainable is represented by the formula: $C_9H_{13}NO_3$.

Abel[1] in his latest publication discusses the analytical differences that exist between the epinephrin of his former papers and the somewhat less altered native principle. He suggests that these differences may be explained by the incomplete saponification of his benzoyl derivative, one benzoyl group being retained, in which case epinephrin would represent the monobenzoyl derivative of the native principle.

It is interesting to note in this connection that if we subtract a benzoyl residue from Abel's formula[2] for "epinephrin" — $C_{17}H_{15}NO_4$ — we obtain a formula $C_{10}H_{10}NO_3$ which is not very far removed from that of adrenalin — $C_9H_{13}NO_3$ — a difference that can be readily explained if we suppose either of the substances to be contaminated with other bodies.

I do not care at the present time to speculate further in regard to this compound, but will leave this for a future communication after the completion of some interesting work that I have well in hand now and which will appear shortly.

I will add in conclusion that although all the chemical contributions to this difficult subject from the time of Vulpian in 1856 until the last few years are of value in directing attention to this body and in discovering certain more or less isolated facts in relation to it the really important contributions have appeared comparatively recently. Before the physical and chemical properties of a substance can be determined definitely and its chemical constitution known it must be isolated in a pure form or some of its derivatives must be obtained pure. Abel has isolated at least the salts of "epinephrin" in an apparently pure form, but in very small quantities; Takamine and myself have isolated what at the present time appears to be the active principle in quantities sufficiently large for an exhaustive investigation.

[1] Abel: Johns Hopkins Hospital Bulletin, 1901, p. 84.

[2] Abel: Zeitschrift für physiologische Chemie, xxviii, p. 325.

STUDIES IN THE PHYSIOLOGY AND PSYCHOLOGY OF VISUAL SENSATIONS AND PERCEPTIONS.

By F. W. ELLIS.

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I. The Duration of Visual Sensations and Perceptions after Momentary Light Impressions.

Introductory and historical. — One of the simplest and most elemental of the problems of vision is the determination of the total duration of luminous sensations. In view of its obvious theoretical importance it is surprising that so little attention has been given to its solution.

It was known to the ancients that the sensation of light persists longer than its cause. Newton conjectured that it might last a second. The first attempt to determine experimentally its duration was probably made by Segner [1] 150 years ago. He made use of the fact, probably known for ages, that a luminous spot moving in a circle with sufficient rapidity appears as a ring of light. By employing a glowing coal, and fixing one part of the luminous ring, he estimated the time required to just complete the circle. I have not access to his original publication, but according to Bosscha [2] he found the necessary time to be $\frac{1}{3}$ second. Helmholtz, [3] however, states that it was $\frac{1}{2}$

[1] Segner: De raritate luminis, Göttingen, 1740.

[2] Bosscha: Graefe's Archiv für Ophthalmologie, 1894, xl, Abtheilung 1, p. 22.

[3] Helmholtz: Physiologische Optik, zweite Auflage, p. 501.

second. To make the discordance of statement greater, Ferry [1] records Segner's result as $\frac{1}{10}$ second. This is doubtless an error, therefore we shall assume that Segner found that the duration of visual sensation was about $\frac{1}{3}$ second.

Twenty-five years later d'Arcy, employing a similar method, found the duration of vision to be 0.13 second. About the beginning of the century Cavallo estimated it to be $\frac{1}{10}$ second. In 1829 Plateau published his inaugural dissertation,[2] in which he records the results of his experiments to determine the time of the persistence of vision. He employed a modification of Segner's method, in which colored papers were substituted for the incandescent coal. He found the time, varying slightly with different colors, to be about $\frac{1}{3}$ second.

It is evident that the attempts of these early experimenters were to discover the total duration of the light sensation after momentary stimulation of the retina. When the sight is immovably fixed upon one spot of the luminous ring in Segner's experiment, every part of the retinal circle corresponding to the ring is momentarily excited once during each revolution of the spot of light. The ring of light will be just complete when the sensation due to the excitation of any element of the retina is vanishing when the next stimulus is received. The rate of revolution of the spot of light necessary for the completion of the luminous circle is the measure of the total duration of the sensation. It must be borne in mind that the total duration is very different from the duration of the maximum sensation. There is some confusion upon this point. The duration of the maximum sensation is revealed in the familiar experiments with rapidly rotating disks, where differently colored or shaded sectors are made to blend into a uniform resultant color. It is well-known that the more intense the illumination of the disk, the shorter the duration of the maximum sensation. Helmholtz likens this to the more rapid loss of temperature in the hotter of two bodies. In the case of the total duration of the sensation it would be natural to expect the converse of this law to be true.

It is remarkable that, until recently, the early experiments that we have mentioned were the only ones on record that have been made to determine the total duration of visual sensations after momentary stimulation. It is equally remarkable that they have been almost

[1] Ferry: American Journal of Science, 1892, xliv, p. 192.

[2] Plateau: Dissertation sur quelques propriétés des impressions produites par la lumière sur l'organe de la vision, 1829.

entirely ignored in the standard text-books. Plateau made a critical summary of his previous work in this direction, after fifty years' devotion to the study of the subjective phenomena of vision, but he does not appear to have altered his original estimate of the duration of visual sensations under the conditions of his experiments. The chief reason for the neglect of these studies is probably the almost universal confusion of the results of momentary stimulation of the visual apparatus with those due to comparatively prolonged action of light. Here the influence of Helmholtz's teaching has been paramount. His view was that after the action of light on the retina ceases, there is a continuation of the primary sensation for a length of time varying largely with the intensity and duration of the illumination. According to him the duration of this primary sensation after the cessation of the stimulus, which he termed the positive after-image, may be seconds, or even minutes after prolonged stimulation. This view was adopted by Fick and other authoritative writers and has been almost universally accepted up to the present time.

Many years ago Aubert [1] discovered that after momentary illumination of objects with an electric spark, there is sometimes a positive complementary after-image following the primary sensation. He also stated that in some of these experiments there is a period of darkness between the primary sensation and its after-image, but that this was not of invariable occurrence. Notwithstanding these observations, his later teaching [2] in regard to the duration of visual sensations was in substantial accord with that of Helmholtz.

Hering became convinced, a considerable time ago, that the prevalent view was not in accordance with facts. At his instigation Hess [3] made an elaborate study of the after-images due to very brief stimulation of the retina. The electric spark was employed as a source of illumination in part of these experiments, but as good results were obtained with a photographic shutter giving exposures of $\frac{1}{100}$ – $\frac{1}{200}$ second. The experiments were all performed in a dark room. Various sources of illumination were used with the shutter arrangement, and in some experiments monochromatic light from a spectral apparatus was employed.

Hess's conclusions were as follows : — Whenever the retina is

[1] Aubert: Moleschott's Untersuchungen, 1858.

[2] Aubert: Graefe und Saemisch's Handbuch der Augenheilkunde, 1876, ii, § 21.

[3] Hess: Archiv für die gesammte Physiologie, 1891, xlix, p. 190.

momentarily excited by light, the primary sensation is of almost immeasurable brevity.

Under certain conditions the primary sensation is followed by a negative or complementary after-image of a duration somewhat less than $\frac{1}{3}$ second.

Then follows a positive after-image lasting ordinarily several seconds; its duration depends on the strength of the stimulus, and the condition of the visual apparatus.

After the disappearance of the positive after-image, a second negative one is sometimes observed.

A very important point abundantly established by these experiments is that after momentary stimulation the positive after-image is not a direct continuation of the primary sensation, but is separated from it by a negative phase. The positive after-image, therefore, is not a part of the primary sensation, but is a reverberation in consciousness, somewhat analogous to an echo.

It is evident that in estimating the duration of a visual sensation, using the term "visual sensation" in the sense in which we ordinarily employ it, we must leave out of account the negative and positive after-images, and concern ourselves only with the primary sensation which precedes the negative phase. According to Hess the primary sensation is extremely brief. He estimated the duration of the combined primary sensation and complementary after-image to be approximately $\frac{1}{3} - \frac{1}{2}$ second. As he also stated that the negative phase alone had a duration of nearly $\frac{1}{3}$ second, we have a right to infer that he regarded the duration of the primary sensation as a very small fraction of a second.

Bosscha[1] repeated Hess' experiments and, in the main confirmed his results. He stated that the duration of the primary sensation was too short to be directly measured, but, by employing an indirect method, he estimated it to be $\frac{1}{10} - \frac{2}{10}$ second. He assumed that Hess by his statement that, in his experiments, the primary sensation disappeared in an almost immeasurably short space of time, meant that it was practically instantaneous, like the electric spark that produced it. Hess emphatically denied this in a later paper.[2] His statements in this article, however, as well as those in the previous one, would indicate that he believed the duration of the primary sensation to be extremely brief.

[1] Bosscha: Graefe's Archiv fur Ophthalmologie, 1894, xl, Abtheilung 1, p. 22.

[2] Hess: Graefe's Archiv für Ophthalmologie, 1894, xl, Abtheilung 2, p. 259.

Object of the research.— The first object of my research was to determine more accurately than had hitherto been done the duration of the primary visual sensation. It is evident that this is the true measure of the persistence of vision. The after-images, ordinarily, have no appreciable effect in consciousness. If they did they would seriously interfere with distinct vision. It is only after relatively prolonged stimulation that they noticeably incommode vision. Here the effects of fatigue assert themselves and complicate the problem. It is not easy to give a definition of visual fatigue; and it is still more difficult to draw the line separating the essential phenomena of every visual act from those due to continuation of the stimulation. Fatigue may pass by insensible degrees into a pathological condition which may be of long duration, or even be permanent. A number of cases are on record of disastrous results to vision from too prolonged exposure to intense light. For the purposes of this paper it will probably be sufficiently accurate to regard visual fatigue as the normal effect of relatively prolonged exposure to light of considerable intensity. The prolonged action of light on the eye may, in a measure, be regarded as the summation of a large number of momentary stimuli, the effect of each one being modified by those preceding and following it. In our attempts to learn as much as possible of the essential phenomena of vision it is desirable to isolate and simplify the problems as much as possible. This is the reason for employing momentary stimulation in studying the duration of visual sensations. The results obtained by this mode of experimentation can be more usefully employed in constructing a theory of vision. Visual fatigue is nearly, if not entirely eliminated from the problem.

Modifications of the Segner experiment.— It is impossible to determine accurately the duration of vision by the subjective methods of Hess. The graphic method is the most accurate one for estimating fractions of a second. We can employ an analogous method in the solution of our problem. Segner's experiment is essentially a graphic one in which records of events taking place at different times are simultaneously exhibited in different parts of the visual field.

It is easy to prove, by repeating his experiment, that Segner was correct in his statement that a visual sensation lasts $\frac{1}{3}$ second, and that later experimenters were wide of the mark in assigning to it a much briefer duration. After a certain amount of practice it can even be demonstrated in this way that the primary visual sensation lasts at least second. No apparatus is required except a glowing

match and a metronome. A piece of incandescent charcoal held in pincers, or any self-luminous object, swung in a circle in the dark, may be employed. A glowing coal is soon extinguished: it is therefore better to use some form of slow match. I have found Chinese joss-sticks useful for this purpose. The light given off from the glowing end of the stick is slight, yet when it is swung in a circle twice in a second in a dark room, the luminous ring is complete and sharply defined. It is very necessary in this and all experiments of similar character to keep the eye fixed upon the same point in space. If the eyes move with the luminous object the circle is no longer complete. The experiment is much more satisfactory if the luminous object is moved by clockwork or some other motor. I have varied the experiment in a great variety of ways. A joss-stick attached to a simple apparatus of variable speed, moved by clockwork has proved itself a very efficient arrangement. A better apparatus is a small incandescent surgical lamp revolved in the same manner. The intensity of the light of the lamp can be varied within wide limits by means of a rheostat. By means of another arrangement offering certain great advantages the retinal image is moved by means of a prism and the object is stationary.

The luminous ring obtained in these experiments varies in intensity in different parts, but does not ordinarily present complementary colors. Its intensity is more uniform when the intensity of the light which causes it is feeble. It will be shown later that the ring as ordinarily obtained, corresponds to the primary sensation, and is not a combination of this with after-images. The after-image is not usually visible under the conditions of the experiment.

Stroboscopic methods of determining the duration of visual sensations. — The Segner experiment is crude and open to various objections. To obviate these objections and to determine more accurately the duration of the primary visual sensation, I have employed various stroboscopic methods which have proven very satisfactory. In these experiments separate portions of the field are stimulated in rapid succession in such a way that comparison of effects is possible, and mutual disturbance reduced to a minimum.

When a small self-luminous body, like a surgical lamp, revolving at the rate of $1\frac{1}{3}$ turns in a second, in a dark room, is viewed through a rapidly revolving stroboscopic disk, a complete ring of separate luminous images is seen. This proves that in this experiment luminous sensations last at least $\frac{3}{4}$ second. After some practice it will

probably be seen that when the object revolves at the slower rate of once a second, the ring of images is nearly, if not quite, complete; showing that Newton was right in his surmise that visual sensations may last about one second. The images fade before they entirely disappear; hence there is some difficulty in determining the exact instant when the circle becomes complete. The estimates of different observers may vary to a slight extent but it is easy to convince one unaccustomed to the experiment that the sensations last fully $\frac{3}{4}$ second. None of the images are after-images; they all correspond to the primary visual sensation, as will be proven later. I have made many experiments of this character. As luminous objects I have employed those already mentioned, as well as spots of solar light reflected from a revolving mirror. By varying the conditions of the experiment it can be easily demonstrated that, within wide limits, variation of the intensity and duration of the stimulus has little, if any, appreciable effect on the duration of the primary sensation.

In order to study more systematically the effect of variation in intensity I devised an apparatus constructed on a different principle. Instead of causing the luminous object to move, I allowed it to remain stationary, and made its image revolve on the retina by means of an apparatus constructed as follows. (See Fig. 1.) A prism of three to ten degrees angle, from an oculist's trial-case, is fastened within a short tube, about 3 cm. in length, which can be made to revolve in an outer tube by means of a pulley and a band passing over a corresponding pulley on the axle of the whirling machine. The prism is made to revolve slowly in front of an adjustable stroboscopic wheel, revolving eight times as fast as the prism. The stroboscopic apparatus is composed of two superposed disks of cardboard, from each of which two diametrically opposite sectors of 45 degrees have been cut. By adjusting the overlapping of the slits the duration of the stimulus can be adjusted within any desired limits. The rate of revolution of the apparatus is timed with a metronome. When the handle of the apparatus is turned once every second, 16 images are thrown upon different parts of the retina during this time. When the apparatus is used to view a small spot of light in a dark room a ring of spots is seen. The intensity of illumination of the spot can be varied in different ways. In order to make comparative measurements of the variations of intensity I adopted the following procedure. Two rooms of my laboratory were made perfectly dark. A small opening covered with ground glass was made in the door separating

them. Diaphragms with holes of various sizes were placed in front of the glass. The opening was illuminated from behind by a candle or lamp fastened to a block sliding on a narrow, graduated board, at right angles to the door. By this arrangement the intensity of illumination of the opening in the diaphragm could be varied to any desired extent, and the variations accurately measured. It can easily be demonstrated in this way that the intensity of the stimulus may be varied within very wide limits without producing any notable change in the duration of the visual sensation. It is possible that a variation

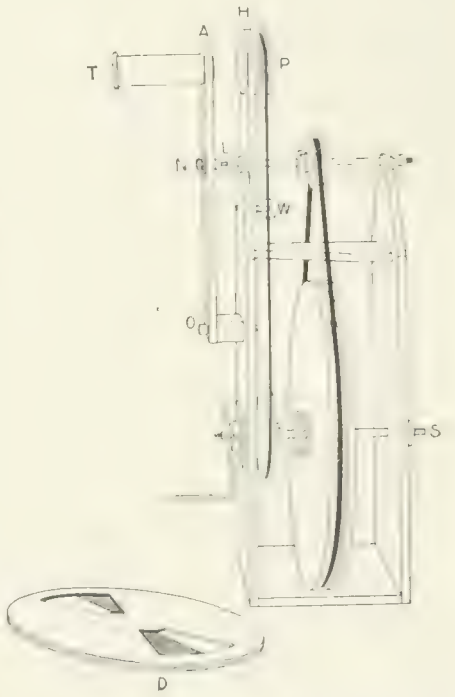


FIGURE 1.—The disks, D, are clamped at L between the nut, N, and the flange on the shaft. The opposite end of the upper shaft also has a screw and flange. The prism, P, revolves in the piece, H, which is clamped to the standard by a screw and the winged-nut, W. The lower end of H has a longitudinal slot, through which passes the upper shaft and the clamping screw: this allows the driving band to be readily tightened, and H to be instantly removed, at any time. The observation tube, T, is held by friction in A, which, in turn, is fastened to the standard by a screw and the nut, O. S is a screw on the end of the lower shaft, by means of which a mirror, disks or other apparatus may be attached.

of a few hundredths of a second might escape detection in these experiments, but no considerable variation would be unnoticed. Just above the threshold of sensation it is possibly somewhat lessened; but this only occurs when the images are so indistinct as to be seen with difficulty. A little above the threshold the duration is as long as with the most intense light employed.

Probably the most accurate way of estimating the duration of the sensation in these experiments is to cause the prism to revolve at such a rate that the ring of images will be just complete in one revolution of the prism. The duration of the sensation will then be the same as the rate of revolution. When the apparatus is turned more slowly, the ring is incomplete. In this case we estimate the duration of sensation by noting the angular space occupied by the images.

The apparatus just described is very convenient for studying the threshold of sensation due to single momentary light impressions. This threshold evidently does not necessarily correspond with that due to the intermittent stimulation experienced when we view the luminous spot through the revolving disk, but not through the prism. In the latter case the retinal area is repeatedly stimulated, but when the spot is seen through the prism, only one stimulus is received on any given area. It is easy to prove with this apparatus that the threshold with momentary stimulation is higher than with intermittent. It is also easy to demonstrate that the threshold varies with the size of the aperture in the diaphragm. With a hole of 5 mm. the threshold is considerably higher than with one of 10 mm.

It is remarkable that, although the threshold is greatly influenced by the intensity and duration of the momentary stimuli, the duration of the resulting visual sensation is little, if at all, affected by them. It is well known that, in many respects, variation of the duration of light impressions is equivalent to corresponding variation in intensity. Having demonstrated that the intensity of momentary stimuli has so little effect on the duration of the primary sensation, it is not surprising to find that variation in the duration of the illumination does not appreciably affect the duration of the sensation. The durations of the stimuli employed were ordinarily within the limits $\frac{1}{10000}$ and $\frac{1}{100}$ second. A duration of $\frac{1}{6000}$ to $\frac{1}{500}$ was most frequently employed.

No observable effect on the duration of the primary sensation was obtained by change in color of the light. The colors were obtained by placing colored glass before the aperture in the diaphragm. In some experiments solar light was used. These experiments will be alluded to later in connection with after-images.

All of the images that we have described are primary images and correspond to the primary sensation. The spacing of the images is regular with different relative rates of revolution of the stroboscopic wheel and the spot of light on the retina, which would not occur if the sensation were discontinuous. So long as the images do not overlap, their size does not influence their duration. It is possible to produce a true after-image in these experiments, which will be described later.

In many of my experiments I employed diffused daylight instead of artificial light, to illuminate the hole in the diaphragm. The opening in the door separating the two rooms in which the experiments were performed is situated directly opposite a window with a northern exposure at a distance of about 4 metres. The windows in

the two rooms are furnished with sliding wooden shutters, by which all light can be excluded. When the room in which the observations are made is darkened, and the shutters of the other room are closed with the exception of the one belonging to the window opposite the door, the opening in the diaphragm appears brilliantly illuminated. By closing this shutter to a greater or less extent, the intensity of the light can be varied within any desired limits.

It is unnecessary that the room should be totally dark in order to obtain good results with the apparatus just described. The experiments can be performed in a partially, or even a well lighted room. The necessary condition is that the whole field of view shall be filled with a uniform background. When the duration of the stimuli is very brief, and the illumination of the room is moderate, the light reflected from a dark background may be insufficient to excite any sensation. If sensation is excited by the background, the efficiency of the light spot as a stimulus to the visual apparatus depends not on its total luminosity, but on its relative brightness, or the difference between its own luminosity and that of the background.

In order to restrict the field of view, and to screen the eyes from extraneous light, I have made an addition to the prism apparatus, which increases its efficiency in many experiments. A tube 10 cm. in length, blackened within, is held by a bracket between the stroboscope and the eye. The axis of the tube coincides with the centre of the prism. The front opening of the tube is covered with a cap having a hole somewhat larger than the pupil of the eye. A piece of black cardboard with a hole in its centre is slipped over the tube. This screens the non-observing eye. Excellent results are obtained with this arrangement in a well-lighted room, if the small opening through which light is transmitted is covered with a large sheet of black or gray cardboard with a small hole in its centre, to serve both as a diaphragm and a background. It is even possible to employ a white background. The white background viewed through the stroboscope will assume some shade of gray; the shade depending on the illumination of the background, the duration of the stimuli, and the number of stimuli per second. Colored backgrounds can also be used.

The experiments that have been described can be easily repeated in any room without any elaborate arrangement of shutters. It is only necessary to interpose a screen of sufficient size, having a narrow opening in its centre, between the prism stroboscope and a window

or lamp. The effect of varying the intensity of the light can be investigated by placing a lamp at different distances behind the screen.

Notwithstanding all these variations in the conditions of the experiment, we find that, when the difference in the luminosity of the background and the transmitted light is sufficient to produce well marked sensation, the duration of the sensation is about $\frac{3}{4}$ second, whatever the intensity and color of the transmitted light and whatever the shade or color of the background may be.

It is also easy to prove with the same arrangement that the sensation of black persists as long as the other visual sensations. The hole in the white background can be made to appear black when no light is transmitted through it. When the black spot is viewed through the rotating prism apparatus a ring of dark spots is seen, and the duration of the images is $\frac{3}{4}$ second.

All these experiments prove, then, that after momentary stimulation, the duration of the visual sensations excited by all kinds of light, and the absence of light, is remarkably constant. This is true when the whole field of view is stimulated with a gray or colored background, as well as when the experiments are performed in a dark room. The sensations probably last a few hundredths of a second longer in the dark room than in the experiments with concomitant illumination of the whole field, but the difference in duration is not marked.

The prism stroboscope can be used to view a great variety of objects, as well as spots of transmitted or reflected light. The objects should be fastened to a background of sufficient size placed near a window, lamp, or other source of light. Small pieces of white or colored paper are most convenient objects. An excellent way to fasten the bits of paper to the cardboard background is to cement them to flat-headed thumb-tacks with sealing-wax. The point of the tack is thrust through the cardboard. This enables the objects to be readily changed, and to be used with the same background. As in our previous experiments, the background may be black, white, gray or colored. It is obvious that any small object that can be fastened to the background may be used in these experiments. If a brilliant spot of light is desired, a highly polished, brass upholsterer's nail answers admirably. The hemispherical head acts as a convex mirror. When a white ground is used there may be a disagreeble flickering, if it is brightly illuminated. This can be obviated by substituting a gray ground. Gray forms an excellent universal back-

ground. White, black, and colored objects are seen equally well upon it. It is necessary, however, that the luminosity of the object should vary in a positive or negative sense, from that of the ground or that the object should be delimited from the ground by shadows. Effects will also vary according to the illumination of the ground. A bit of colored paper of the same luminosity as the gray ground, may be seen with difficulty, or not at all, when the duration of the stimulus is very brief, and the illumination weak. Under the same circumstances, the color of the same object may be very apparent on a white, or black ground; showing that difference in the luminosity of the object and ground is a very important factor in determining the visibility of objects in these experiments.

Whatever the object or background employed may be, when they are viewed through the prism apparatus making $1\frac{1}{3}$ turns per second, it will be found that the images last nearly, or quite $\frac{3}{4}$ second, provided the object is readily seen.

An experiment that proves in a somewhat striking manner that the duration of the sensation is not dependent upon the intensity of the light causing it is the following. The background was a very dark gray, which would ordinarily be called black. A small piece of the same cardboard was cemented to a thumb-tack, and fastened to the centre of the ground. The edges of the object were blackened with india ink. The thickness of the object caused it to be bordered with shadows when the background was placed in a good light, near a window. The duration of the images was found to be $\frac{3}{4}$ second, notwithstanding the slight difference in luminosity between the object and the shadows.

We can vary these experiments by fastening the object to a disk rotated by clockwork, and employing an ordinary stroboscope without the prism. It can be proven that the sensations excited by the rotating object may persist about $\frac{3}{4}$ second. The best results are obtained with small objects placed midway between the centre of the disk and its edge. The disk should be of uniform shade and color. The centre of the disk should be fixed with the sight and the eye should be restrained from its tendency to follow the movements of the object. The ring of images should not occupy too much of the field. It must be remembered that some of the images are extremely faint, and that there is consequently a tendency of the mind to ignore them. For reasons that will appear later it is necessary that the disk should make about 80 turns per minute.

It is easy to demonstrate and measure the persistence of vision without the prism apparatus, clockwork, or any motor. For this purpose it is necessary to have a whirling machine with two parallel shafts. The handle is attached to one end of the lower shaft, and a disk of cardboard bearing the object is fastened to the other end. The upper shaft, which is made to revolve rapidly by multiplying pulleys, carries the stroboscopic disks. The whole apparatus is placed before a mirror. The image of the revolving object is viewed in the mirror through the stroboscope.

These stroboscopic experiments can be varied in many ways, all demonstrating in the most convincing manner that the duration of visual sensations after momentary light impressions is longer than has hitherto been supposed, and that this duration is only slightly affected by variations in the intensity of the stimuli, contrary to what has been previously taught.

Effect of psychological factors on the duration of visual sensations. — The only factors that seem to have a marked effect on the duration of the sensations are psychological ones. There appears to be a tendency of the mind to ignore other visual sensations, when the attention is especially drawn to any particular sensation or perception. If the sensations that are disregarded are faint they may entirely disappear from consciousness. If the sensations are well-marked there is more difficulty in inhibiting them. We find that the rate of revolution of an object may affect the duration of the visual sensations excited by it. This is strikingly proven by the following experiment. Two black disks are placed side by side, and are made to turn by clockwork, one at the rate of 40, and the other at the rate of 80 revolutions per minute. A small piece of white or colored paper is fastened at the same distance from the centre on each disk. When the revolving disks are viewed through a stroboscope, and the sight is fixed alternately upon one and then upon the other, it will be seen that the images cover a complete circle upon the more rapidly revolving disk, but occupy no more than a quadrant upon the slower disk. If we estimate the duration of the sensation with the slower rate of turning we shall probably make it less than half that previously found. It is difficult to explain satisfactorily these discordant results unless we ascribe them to psychological causes. The slower the rate of revolution of the object, the greater the tendency to follow it with the eye; but movements of the eye do not explain the effect of the variation of the rate of revolution. When the eye is kept immovable, the

discrepancy in results still persists. When the object revolves forty times per minute, it is possible to follow it with the attention when the eye is fixed. There is a tendency under these circumstances, to suppress some of the images and to fix the attention exclusively upon the first and relatively brightest ones. When the rate of revolution is increased to sixty and above, it becomes more difficult, or impossible, to follow the object with the attention ; there is, consequently, no longer the same tendency to suppress the images. If this explanation is correct, it is evidently necessary to employ a rate of revolution that just causes a complete circle of images in order to estimate the duration of luminous sensations.

With the prism stroboscope the effect of varying the rate of revolution of the retinal image upon the duration of the sensation is frequently less apparent than in the experiments with rotating disks, and under some circumstances may be wanting. When the apparatus is used in a dark room, and the light is relatively intense, the apparent duration of the sensations may be the same with all rates of revolution of the prism. In this case it would seem that all the images are so vivid that none are suppressed. With a rate of revolution less than sixty per minute much of the success of the experiment depends upon practice.

When other conditions remain the same, all visual sensations are affected alike by variation in the angular velocity of the images. When the object disk makes forty revolutions in a minute, sensations excited by black, white, and variously colored objects persist for the same length of time. A series of comparative experiments which prove this statement was made as follows. Upon a gray disk, revolving forty times per minute, two small objects were fastened, one directly under the other in the same radial line. The objects were of different color and luminosity. It was found when they were both viewed through a stroboscope, that both sets of images occupied the same angular space, and consequently had the same duration. White and black, and various colors were compared with one another, and were proven in this way to excite sensations of the same duration.

Up to this point we have employed the term visual sensation rather loosely. A visual sensation unassociated with other sensations or ideas is an abstraction, and not a real fact of consciousness. The nearest approach to a simple visual sensation would be excited by light of uniform color and intensity filling the entire visual field. Under all ordinary circumstances the field is mapped out into areas

of light and shade, and varying colors. We distinguish form, as well as quality and intensity of light. We recognize objects, and assign them a definite position with reference to other objects. In a word, we perceive objects, as well as recognize the qualities of the light coming from the objects. Perceptions, undoubtedly, involve more complicated processes in the nervous centres than those to which simple sensations are due. They bring into activity higher centres than those concerned with sensation alone. It is obvious that we have been studying the duration of visual perceptions in the experiments that have been described. However, these perceptions cannot be disassociated from the sensations that give rise to them. The sensations must persist as long as the perceptions. When we see a red object the sensation of redness must persist as long as the perception of the object; otherwise we should cease to be conscious of a *red* object, and the perception would change. For this reason it seems to be ordinarily unnecessary to make a distinction between the duration of visual sensations and visual perceptions. The point to be emphasized is that the sensations excite perceptions that involve complicated processes in the brain that require a considerable time for their development, and that when the light impressions are brief, the length of time required is very constant. However brief or slight the stimulus, if it be sufficient to affect consciousness, it sets in operation a train of physiological processes which, if they be allowed to run their full course, last the greater part of a second.

It has already been shown that the rate of revolution of an object may affect the duration of the perception of the object. The duration may be affected by other psychological causes. If in place of a small round, square, or oblong object placed at some distance from the centre of a disk rotating eighty times per minute, we employ a narrow sector with its point terminating in the centre, we shall find it difficult to see that the images cover an entire circle. When the sector is displaced outwards, so that its point describes a circle, the difficulty is lessened or disappears. The experiment may be varied as follows. The base of a narrow triangle of paper or cardboard is cemented to a thumb-tack. The altitude of the triangle should be less than half the radius of the disk. The point of the tack is thrust through the disk and a piece of cork on its opposite side. If the disk revolves eighty times per minute, and the point of the triangle terminates in its centre, it will seem to most observers viewing the object through a stroboscope that the images cover only a part of a

circle, although the tendency to underrate the number of images may sometimes be overcome. If the triangle is then rotated 180° about its base, so that it now points to the periphery, it will be very apparent that the images extend entirely around the circle. In order to study most successfully the duration of visual sensations it is necessary to be able to concentrate the mind upon them, and to exclude other perceptions, so far as possible. This can be best accomplished by surrounding the object with a uniform background. When the narrow sector extends from the periphery to the centre of the disk, and the stroboscope revolves rapidly it is difficult to make the images cover the whole circle. If the number of images be reduced by diminishing the rate of revolution of the stroboscope, they can much more easily be made to extend around the circle.

It appears sufficiently demonstrated from our experiments that the influences which affect the duration of visual perceptions are largely subjective. The duration of the perception depends very little upon the quality and intensity of the light which excites the sensations giving rise to the perception, but it may be abridged by the interference of other perceptions, or by agencies affecting the working of the cerebral centres concerned with vision.

II. The Sensation of Black, and the Independence of Visual Perceptions.

It has been demonstrated in our experiments that black persists as long as other visual sensations; but this fact alone sheds little light on the question of the true nature of the sensation of black. Psychologically considered, we must concede that black is an independent sensation. The question that interests the physiologist is whether the psychological state is due to physiological processes in the visual organs, or the absence of such processes; whether, in other words, we are to believe that black is a negative sensation due to lack of excitation in the corresponding part of the visual field, or whether it has its origin in physical or chemical changes in a visual substance. It is impossible to measure directly the duration of a simple visual sensation alone: we can only measure the duration of the sensation in the perception into which it enters. The duration of the sensation is governed by that of the perception. The sensation can be prevented or suppressed if it interferes with the perception. When we view through a stroboscope a black object rotating

on a white or gray ground, we experience more than a number of simple sensations of black; we *perceive* a number of black objects definitely located in space. Perception of the dark object is the dominant and necessary thing. Sensations which interfere with the perception are suppressed. At first thought it might appear that we are only studying the duration of the white light of the disk in this experiment, but a little consideration will prove that this is not the case. If there is a complete circle of images most of them will be black and distinct. We will call the image in advance, which is the most recent one, No. 1. This image falls on a portion of the field that has been repeatedly stimulated, at very short intervals, by the white light of the disk. The white sensation should persist here with almost its full intensity if not interfered with; but the white sensation is immediately inhibited and replaced by the black. There is no fusion of the two sensations, for the image is not gray. At the passage of the next slit before the eye, Image No. 1 becomes No. 2, and a new image is formed in front of it. When the next slit passes, No. 2 becomes No. 3; and so on until the image finally disappears from consciousness. While the image persists it is illuminated at each passage of an opening in the stroboscope by the white light of the disk. The black image persists, notwithstanding that the portion of the field which it occupies is repeatedly stimulated by the white light. The persisting sensation of black prevents the development of the sensation of white; or perhaps it would be more accurate to say, the persisting perception suppresses the white sensation. There is here a conflict of sensations or perceptions in the monocular field.

The proof that there is no immediate fusion of the sensations excited by the object and the background is more striking when we employ a colored object on a differently colored ground. A narrow sector of blue on a red ground appears with its original blue color when rotated and viewed through a stroboscope. The blue and the red do not combine in the images to form a purple, notwithstanding that the two colors fall upon the same spot on the retina in rapid succession. The only change in the colors is their lessened luminosity due to the stroboscope. Whatever the colors of the object and ground may be, it is found that they do not combine in the images. Here again sensations are inhibited or suppressed by perceptions. It is interesting to contrast these results with those derived from the familiar experiment with the color wheel. When a disk with variously colored sectors is very rapidly rotated, the colors blend into a

uniform composite color differing from any of its constituents. The sectors revolve so rapidly that they do not make any individual impression on the mind. We do not perceive the sectors, we only perceive the disk as a whole. There is no conflict of perceptions here. The sensation excited by each sector is free to fuse with those due to the other sectors, without affecting the distinct perception of the whole disk. Were we able to determine the total duration of the sensations excited by the sectors, we should probably make the nearest approach possible to the determination of the duration of simple visual sensations unaccompanied by distinct perception.

III. The Conflict of Perceptions Due to the Overlapping of Images.

Up to this point we have been careful in our experiments to prevent any overlapping of images. We will now attempt to discover what takes place when the images are not entirely separate. If the images exactly coincide, the effect of their superposition is simply to increase the luminosity of the resultant image. This happens when we view a stationary object through the stroboscope. If the images are displaced in any way so that they no longer coincide, a conflict of visual perceptions becomes strikingly apparent. Each image seeks to delimit itself at the expense of its neighbor. We can readily produce the overlapping of images by viewing through a stroboscope a sector of some size upon a slowly revolving disk. If the sector is white and the ground is black, we shall find that the overlapping borders of the images are limited by dark areas in the form of sectors. The dark bands might, at first, be taken for contrast effects, but a modification of the experiment demonstrates that contrast, as the term is ordinarily understood, does not explain them. If the ground is colored the limiting sectors have more or less of the color of the ground. The explanation of this appearance would seem to be that each image inhibits a portion of the overlapping image in the adjacent field. This serves to delimit the image and to make its individual impression stronger. It is to be especially noted that although the overlapping images are suppressed in the limiting areas, sensibility is not wanting here. The light of the ground is able to excite more or less of its proper sensation in these areas. The sensation excited by the ground is not inhibited in the portions of the images corresponding to the limiting areas, as it would be if the

images did not overlap. A glance at the diagram will make our explanation more intelligible.

The diagram represents two overlapping images in the subjective field. The sector bordered with full lines represents the latest image, which we will call No. 1. The broken lines limit an older image, No. 2. Many more images are seen in the experiment, but, to simplify the explanation, only two are represented. The disk is supposed to be rotating in the direction of the arrows which indicate the angular width of the overlapping images. The distance from the anterior full line to the anterior broken line represents the amount of overlapping of the images. Sectors having more or less of the color of the ground are represented at *a* and *b*. The sector at *a* is due to the inhibitory effect of the border of Image No. 2 on Image No. 1. The color of No. 1 is less marked or wanting here, and the color of the ground asserts itself. Sector *b* is due to the inhibitory effect of Image No. 1 on No. 2. A part of No. 2 is wholly or partly suppressed, and the sensation due to the ground takes its place.

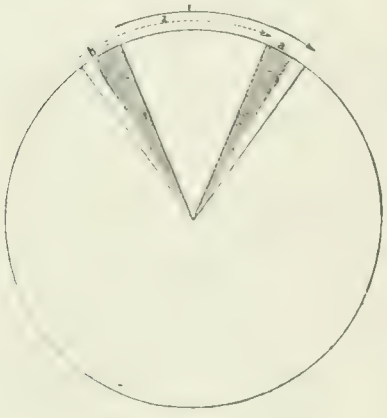


FIGURE 2.

A fact of great importance to be noted is that, however much or little the images overlap, their edges are always sharply defined and appear with their proper color; in other words the inhibitory area never extends entirely to the edge of the image in which it falls. This would seem to indicate that the phenomenon is a psychological one, rather than one due to physiological changes in the retina. If we were to suppose that when the image of an object or spot of light falls upon the retina, an adjacent zone in the retina becomes less sensitive, we should expect that the zone would remain of the same width when the conditions of the experiment remain substantially the same. If the zones remained of the same width with different amounts of overlapping of images, the edge of the overlapping image should sometimes fall within the zone and consequently be less clearly seen. As this never occurs in our experiments, we conclude that the phenomenon which we are studying cannot be ascribed to changes in the retina, but is due to the activity of the higher visual centres. We have here an instance of the conflict of perceptions or an effort of the mind to prevent fusion of images.

We may allude, in this connection, to another effect of the over-

lapping of images. If we view a small spot on a disk rotating forty to sixty times per minute, and gradually increase the number of images seen through the stroboscope, the images will overlap when they become sufficiently numerous. The train of images becomes shortened at the moment of overlapping. In this observation we have another proof of the influence of psychological factors on the duration of visual perceptions. The extreme importance of these psychological factors in the processes of vision has been more and more clearly demonstrated in this series of researches. The physiology of vision is something more than the physiology of the retina; it is largely an important section of the physiology of the brain.

IV. After-Images due to Momentary Stimulation of the Retina.

The after-image and the negative period. — After-images have not ordinarily been noted in the experiments that I have hitherto described, but by modifying those performed in the dark room they may be made to appear. It is very easy to prove, what has been especially insisted upon by Hess,[1] that the positive after-image which follows momentary stimulation is not a direct continuation of the primary image, but is separated from it by a period of darkness. The room in which the experiments are performed should be absolutely dark, and the eyes should be made more sensitive by the exclusion of light for a period. A small piece of incandescent carbon, or the glowing end of a recently extinguished match, when slowly moved about in the field of vision is followed by a trail of light, representing the primary image. This is followed at some distance by the after-image in the form of another trail of light of much less intensity, separated from the first one by a period of darkness. Hess[2] substituted with great advantage a small electric lamp for the burning coal. Bidwell[3] employed patches of solar light reflected upon a screen by means of a slowly revolving mirror forming a small angle with the perpendicular to the axis of rotation.

The clearness with which the after-image is seen depends upon the intensity of the stimulus and the adaptation of the retina. The duration of the after-image also depends upon the intensity of the light,

[1] Hess: Archiv für die gesammte Physiologie, 1891, xlix, p. 190.

[2] Hess: Graefe's Archiv für Ophthalmologie, 1894, xl, Abtheilung 2, p. 268.

[3] Bidwell: Nature, 1894, l, p. 466.

and ordinarily amounts to several seconds. The image may not be noticed at all after very feeble momentary stimuli. When the stimulus is sufficiently intense the after-image shows at first some of the color of the primary image, but afterwards becomes nearly or quite colorless. The primary coloration of the after-image has been denied by von Kries and others, but it has been proven to exist, in some experiments at least, by Hess.[1] I have repeated Hess's experiments with a small incandescent lamp viewed through colored glass, and am able to confirm his statements regarding the coloration of the after-image. The primary coloration is well shown when an intense red light is used, notwithstanding it has been stated by Bidwell that red is never followed by an after-image.

The intensity of the after-image compared with that of the primary image is very slight indeed. This is proven by the fact that it is only visible in a perfectly dark room, and does not affect consciousness at all in ordinary vision. It may be termed the visual echo.

The negative period dividing the primary from the after-image is of constant occurrence. It is undoubtedly a period of diminished sensibility, but it is probable that the diminished sensibility following the primary image is of longer duration than the negative period separating the two images. The following experiment shows very clearly that the sensibility of the visual elements is affected for a considerable time after very brief stimulation of moderate intensity. A narrow white sector on a black disk is made to revolve at the rate of forty turns per minute, near a lamp, in a darkened room. If the revolving disk is viewed through a stroboscope placed as near as possible without obstructing the illumination, the white images are seen to be followed by a series of black sectors on the dark gray ground of the disk. The disk reflects a certain amount of light. The visual elements that have been stimulated to give rise to the primary images are less sensitive to this feeble reflected light; they do not respond to it, while the elements of the surrounding field are slightly stimulated, giving rise to the appearance of the dark sectors. The duration of the dark images is greater than that of the white ones; in some experiments it was twice as great.

In the dark room experiments with the prism stroboscope that I have detailed, the feeble after-images are not visible in the presence of the relatively intense primary images. I have modified the ex-

[1] Hess: Graefe's Archiv für Ophthalmologie, 1897, xliv, p. 445.

periments so as to make the after-images visible. A photographic "time" shutter, operated by a pneumatic arrangement was mounted in front of the stroboscopic disks, in place of the observation tube. The shutter was adjusted to open and shut in a little less time than that required for a complete revolution of the prism. With this arrangement brief glimpses of the circle of primary images could be obtained before the appearance of the after-images. When the stroboscope was revolving at an appropriate rate in the dark room, the bulb of the shutter was pressed, and an incomplete ring of primary images was seen, followed in a short space of time after the closure of the shutter by the after-images. The after-images were usually faint and nearly or quite colorless. If the light employed was of very slight intensity, and the stimulus very brief, the after-images were sometimes wanting. The after-images could be much better observed by dispensing with the stroboscopic disks and viewing the light spot through the shutter and revolving prism alone. The image of the spot was thus elongated to a curved band of light. This forms a very convenient arrangement for observing and controlling the phenomena of the after-images. In this way the intensity and color of the light can be easily varied. The after-images can be satisfactorily observed without the photographic shutter. If a screen with an opening two or three centimetres in diameter is placed between the prism and the eye, it is easy to place the head and the apparatus in such positions that, by revolving the prism once, the curved band of light can be made to sweep through the field and disappear, leaving the field sufficiently dark to enable the after-images to be studied.

The negative period was found in these experiments to be of constant occurrence. In some instances it appeared to be as long as the duration of the primary image, but there was no systematic attempt to measure it under varying conditions, although it is desirable that such experiments should be made.

I have noted in these and other experiments that the size of the after-image is not always the same as that of the primary image. The after-image is frequently much more diffuse, covering a larger part of the field. This may indicate that when a portion of the visual field is stimulated an adjacent zone is affected in some way, and that this process in the outlying zone gives rise to an after-image.

Changes in the color of the primary image. — Hess asserts that, under favorable conditions, the original color of the primary image changes

to a complementary one before the period of darkness separating the primary from the after-image. It seemed desirable to test the correctness of this statement with the stroboscope. If the primary image changes in color during its persistence, the images seen through the stroboscope should vary in color. If, for instance, a green image changes to a complementary purple, the stroboscopic images in advance should be green, and the older ones purple. In most of my stroboscopic experiments a change to a complementary color was not apparent. When the light which occasions the primary image is of moderate intensity the original color persists nearly or quite as long as the image. Objects luminous by reflected light, such as pieces of colored paper, fastened to a revolving black disk, and viewed through an ordinary stroboscope, do not show the complementary change in coloration. This is also true when the objects are placed on a stationary ground and viewed through the prism stroboscope. The images of a small illuminated aperture do not usually show any complementary colors, although when the light is intense, and other conditions are favorable, a change of color, more or less complementary, may sometimes be noted. The ordinary changes in coloration are what might be more properly ascribed to fading. Black and white images tend to become grayish just before their disappearance, and this is also true of faintly colored images of feeble luminosity. In my experiments complementary changes in coloration have been best seen with intense blue light. When a small aperture covered with blue glass, through which reflected sunlight is transmitted, is viewed through the prism stroboscope in a perfectly dark room, if the light is sufficiently intense most of the blue primary images, especially the older ones, will be seen to be surrounded by a yellowish zone or corona. The area of the primary image is thus considerably enlarged. The true primary image is the central blue one; the yellowish halo is an induced image. As the blue images fade, the yellow zones become relatively more conspicuous, and may finally spread over the blue images and obliterate them entirely. If the light is less intense, the central blue image may persist as long as the yellow zones. When the intensity of the light becomes moderate, the yellowish halo disappears. I have been less successful in inducing a complementary color with green. Under especially favorable circumstances the stroboscopic green images may become slightly pink before they disappear. Little if any change of color is noticeable with yellow light, and ordinarily

none is observed with red. When the red light is very intense, however, the older images may become somewhat blue, but I have never noticed any green coloration in them.

In order to detect changes in the coloration of the images with the prism stroboscope, the experiment may be conducted as follows: — The observation tube and its support are removed. If the head is held in the right position before the disks, a stationary image is seen, as well as the ring of revolving images. One eye is directly in front of the prism, and the circle of images is seen with this eye. The stationary image is seen by the other eye, which looks through the disks, but not through the prism. As the stationary image is due to repeated stimulation at short intervals, it is a constant one, and is ordinarily brighter than those of the circle which are due to a single stimulus. The color of the various images in the circle may thus be conveniently compared with that of the fixed image. This method of using the two eyes can sometimes be usefully employed in other stroboscopic experiments, as it affords a fixed point, by regarding which the eyes can be held steadily in one position, and can be more readily focussed upon the object.

In some of the experiments made in studying the changes in coloration of the primary images, I employed the photographic shutter before the stroboscope, in the manner already described. The results obtained were substantially the same as those observed with the less complicated apparatus.

I have been able, therefore, only partially to confirm Hess's statements regarding complementary images, by means of the methods which I have employed. According to my experience the complementary changes in color require specially favoring circumstances, and do not occur after all colors. I prefer, therefore, not to speak of a separate complementary image. The color of the primary image may change, but the change is more or less gradual. It would be hard to say where the line should be drawn separating the images according to their hue or tint. Under ordinary circumstances the changes are slight, or are wanting. For these reasons I prefer to distinguish only the primary image, and the after-image which is separated from it by the negative period. It is, of course, an error to regard the duration of a color sensation as co-extensive with that of the primary image, if the color of the image varies. However, we have found that under the conditions of ordinary vision, the color persists unchanged nearly, if not quite, as long as the primary image.

Summary.

1. Under favorable conditions the duration of visual sensations and perceptions due to momentary light impressions is about $\frac{3}{4}$ second; and this duration is only slightly affected by variation of the intensity or color of the light employed.

2. The factors which chiefly affect the duration of visual sensations and perceptions are psychological.

3. After the disappearance of the primary image due to momentary stimulation of the visual apparatus, the sensibility of the affected portion of the visual field is lessened for a considerable fraction of a second.

4. When a portion of the subjective visual field is occupied by a visual perception, the perception does not fuse with sensations subsequently excited in the same portion of the field.

5. A visual image can inhibit a portion of an overlapping image in order to prevent fusion of the images.

6. The color of the primary image does not ordinarily change, to any great extent, during the persistence of the image.

7. If the light is intense the color of the primary image may change, but the change of color is not always complementary.

8. With certain colors, especially blue, a complementary change of color in the primary image may be noted.

9. After very brief illumination of the retina, if the field remains perfectly dark, and the light is sufficiently intense, the primary image is followed by an after-image, after a period of darkness of considerable length.

10. The intensity of the after-image is very slight in comparison with that of the primary image.

11. The size of the after-image does not always correspond to that of the primary image.

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